Baskar
09/833017

Seq. IDs 244

```
     FILE 'REGISTRY' ENTERED AT 14:48:46 ON 13 NOV 2002
L1             10 SEA ABB=ON  PLU=ON  MKKTLSLKNDFKEIKTDELEIIIGGSGSLSTFFRLFN
                  RSFTQALGK|SGSLSTFFRLFNRSFTQALGK/SQSP

L1   ANSWER 1 OF 10  REGISTRY  COPYRIGHT 2002 ACS
RN   439068-12-9  REGISTRY
CN   13: PN: US20020081302 SEQID: 13 unclaimed protein (9CI)  (CA INDEX
     NAME)
CI   MAN
SQL  46

SEQ      1 MKKTLSLKND FKEIKTDELE IIIGGSGSLS TFFRLFNRSF TQALGK
           ========== ========== ========== ========== ======
HITS AT:   1-46

**RELATED SEQUENCES AVAILABLE WITH SEQLINK**

REFERENCE   1:  137:62148

L1   ANSWER 2 OF 10  REGISTRY  COPYRIGHT 2002 ACS
RN   439068-11-8  REGISTRY
CN   12: PN: US20020081302 SEQID: 12 unclaimed protein (9CI)  (CA INDEX
     NAME)
CI   MAN
SQL  46

SEQ      1 MKKTLSLKND FKEIKTDELE IIIGGSGSLS TFFRLFNRSF TQALGK
           ========== ========== ========== ========== ======
HITS AT:   1-46

**RELATED SEQUENCES AVAILABLE WITH SEQLINK**

REFERENCE   1:  137:62148

L1   ANSWER 3 OF 10  REGISTRY  COPYRIGHT 2002 ACS
RN   439068-10-7  REGISTRY
CN   11: PN: US20020081302 SEQID: 11 unclaimed protein (9CI)  (CA INDEX
     NAME)
CI   MAN
SQL  46

SEQ      1 MKKTLSLKND FKEIKTDELE IIIGGSGSLS TFFRLFNRSF TQALGK
           ========== ========== ========== ========== ======
HITS AT:   1-46

**RELATED SEQUENCES AVAILABLE WITH SEQLINK**

REFERENCE   1:  137:62148

L1   ANSWER 4 OF 10  REGISTRY  COPYRIGHT 2002 ACS
RN   439068-08-3  REGISTRY
CN   9: PN: US20020081302 SEQID: 9 unclaimed protein (9CI)  (CA INDEX
     NAME)
CI   MAN
SQL  46

SEQ      1 MKKTLSLKND FKEIKTDELE IIIGGSGSLS TFFRLFNRSF TQALGK
           ========== ========== ========== ========== ======
```

HITS AT: 1-46

RELATED SEQUENCES AVAILABLE WITH SEQLINK

REFERENCE 1: 137:62148

L1 ANSWER 5 OF 10 REGISTRY COPYRIGHT 2002 ACS
RN 439068-07-2 REGISTRY
CN 8: PN: US20020081302 SEQID: 8 unclaimed protein (9CI) (CA INDEX NAME)
CI MAN
SQL 46

SEQ 1 MKKTLSLKND FKEIKTDELE IIIGGSGSLS TFFRLFNRSF TQALGK
========== ========== ========== ========== ======
HITS AT: 1-46

RELATED SEQUENCES AVAILABLE WITH SEQLINK

REFERENCE 1: 137:62148

L1 ANSWER 6 OF 10 REGISTRY COPYRIGHT 2002 ACS
RN 439068-06-1 REGISTRY
CN 7: PN: US20020081302 SEQID: 7 unclaimed protein (9CI) (CA INDEX NAME)
CI MAN
SQL 46

SEQ 1 MKKTPSLKND FKEIKTDELE IIIGGSGSLS TFFRLFNRSF TQALGK
===== ========== ======
HITS AT: 26-46

RELATED SEQUENCES AVAILABLE WITH SEQLINK

REFERENCE 1: 137:62148

L1 ANSWER 7 OF 10 REGISTRY COPYRIGHT 2002 ACS
RN 439061-07-1 REGISTRY
CN L-Lysine, L-methionyl-L-lysyl-L-lysyl-L-threonyl-L-leucyl-L-seryl-L-leucyl-L-lysyl-L-asparaginyl-L-.alpha.-aspartyl-L-phenylalanyl-L-lysyl-L-.alpha.-glutamyl-L-isoleucyl-L-lysyl-L-threonyl-L-.alpha.-aspartyl-L-.alpha.-glutamyl-L-leucyl-L-.alpha.-glutamyl-L-isoleucyl-L-isoleucyl-L-isoleucylglycylglycyl-L-serylglycyl-L-seryl-L-leucyl-L-seryl-L-threonyl-L-phenylalanyl-L-phenylalanyl-L-arginyl-L-leucyl-L-phenylalanyl-L-asparaginyl-L-arginyl-L-seryl-L-phenylalanyl-L-threonyl-L-glutaminyl-L-alanyl-L-leucylglycyl- (9CI) (CA INDEX NAME)
OTHER NAMES:
CN 2: PN: US20020081302 SEQID: 1 claimed protein
CN Competence signal peptide (Streptococcus mutans)
CI MAN
SQL 46

SEQ 1 MKKTLSLKND FKEIKTDELE IIIGGSGSLS TFFRLFNRSF TQALGK
========== ========== ========== ========== ======
HITS AT: 1-46

RELATED SEQUENCES AVAILABLE WITH SEQLINK

REFERENCE 1: 137:62148

L1 ANSWER 8 OF 10 REGISTRY COPYRIGHT 2002 ACS
RN 438620-89-4 REGISTRY
CN L-Lysine, L-serylglycyl-L-seryl-L-leucyl-L-seryl-L-threonyl-L-phenylalanyl-L-phenylalanyl-L-arginyl-L-leucyl-L-phenylalanyl-L-asparaginyl-L-arginyl-L-seryl-L-phenylalanyl-L-threonyl-L-glutaminyl-L-alanyl-L-leucylglycyl- (9CI) (CA INDEX NAME)
OTHER NAMES:
CN 14: PN: US20020081302 SEQID: 14 unclaimed sequence
SQL 21

SEQ 1 SGSLSTFFRL FNRSFTQALG K
========== ========== =
HITS AT: 1-21

REFERENCE 1: 137:62148

L1 ANSWER 9 OF 10 REGISTRY COPYRIGHT 2002 ACS
RN 330647-52-4 REGISTRY
CN L-Lysine, L-methionyl-L-lysyl-L-lysyl-L-threonyl-L-leucyl-L-seryl-L-leucyl-L-lysyl-L-asparaginyl-L-.alpha.-aspartyl-L-phenylalanyl-L-lysyl-L-.alpha.-glutamyl-L-isoleucyl-L-lysyl-L-threonyl-L-.alpha.-aspartyl-L-.alpha.-glutamyl-L-leucyl-L-.alpha.-glutamyl-L-isoleucyl-L-isoleucyl-L-isoleucylglycylglycyl-L-serylglycyl-L-seryl-L-leucyl-L-seryl-L-threonyl-L-phenylalanyl-L-phenylalanyl-L-arginyl-L-leucyl-L-phenylalanyl-L-asparaginyl-L-arginyl-L-seryl-L-phenylalanyl-L-threonyl-L-glutaminyl-L-alanyl-L-leucylglycyl- (9CI) (CA INDEX NAME)
OTHER NAMES:
CN Competence-stimulating protein (Streptococcus mutans strain GB14 gene comC)
CN GenBank AF277152-derived protein GI 12698430
CI MAN
SQL 46

SEQ 1 MKKTLSLKND FKEIKTDELE IIIGGSGSLS TFFRLFNRSF TQALGK
========== ========== ========== ========== ======
HITS AT: 1-46

RELATED SEQUENCES AVAILABLE WITH SEQLINK

REFERENCE 1: 134:277785

L1 ANSWER 10 OF 10 REGISTRY COPYRIGHT 2002 ACS
RN 330647-51-3 REGISTRY
CN L-Lysine, L-methionyl-L-lysyl-L-lysyl-L-threonyl-L-prolyl-L-seryl-L-leucyl-L-lysyl-L-asparaginyl-L-.alpha.-aspartyl-L-phenylalanyl-L-lysyl-L-.alpha.-glutamyl-L-isoleucyl-L-lysyl-L-threonyl-L-.alpha.-aspartyl-L-.alpha.-glutamyl-L-leucyl-L-.alpha.-glutamyl-L-isoleucyl-L-isoleucyl-L-isoleucylglycylglycyl-L-serylglycyl-L-seryl-L-leucyl-L-seryl-L-threonyl-L-phenylalanyl-L-phenylalanyl-L-arginyl-L-leucyl-L-phenylalanyl-L-asparaginyl-L-arginyl-L-seryl-L-phenylalanyl-L-threonyl-L-glutaminyl-L-alanyl-L-leucylglycyl- (9CI) (CA INDEX NAME)
OTHER NAMES:
CN Competence-stimulating protein (Streptococcus mutans strain BM71

gene comC)
CN GenBank AF277151-derived protein GI 12698428
CI MAN
SQL 46

SEQ 1 MKKTPSLKND FKEIKTDELE IIIGGSGSLS TFFRLFNRSF TQALGK
===== ========== ======

HITS AT: 26-46

RELATED SEQUENCES AVAILABLE WITH SEQLINK

REFERENCE 1: 134:277785

FILE 'HCAPLUS' ENTERED AT 14:51:25 ON 13 NOV 2002
L2 2 S L1

L2 ANSWER 1 OF 2 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2002:488061 HCAPLUS
DOCUMENT NUMBER: 137:62148
TITLE: Signal peptides, nucleic acid molecules and methods for treatment of caries
INVENTOR(S): Cvitkovitch, Dennis; Lau, Peter C. Y.; Li, Yung Hua
PATENT ASSIGNEE(S): Can.
SOURCE: U.S. Pat. Appl. Publ., 50 pp.
CODEN: USXXCO
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 2002081302	A1	20020627	US 2001-833017	20010917
CA 2332733	AA	20011010	CA 2001-2332733	20010220

PRIORITY APPLN. INFO.:
CA 2000-2302861 A 20000410
CA 2001-2332733 A 20010220
US 2001-269949P P 20010220

AB The invention relates to a compd. that competitively inhibits binding of competence signal peptide (CSP) to Streptococcus mutans histidine kinase. The compd. is preferably a peptide or an antibody. The compd. is preferably a deriv. of [SEQ ID NO:2], a fragment of [SEQ ID NO:2] or a deriv. of a fragment of [SEQ ID NO:2].

IT **439061-07-1**
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(amino acid sequence; signal peptides, nucleic acid mols. and methods for treatment of caries)

IT **439068-06-1 439068-07-2 439068-08-3 439068-10-7 439068-11-8 439068-12-9**
RL: PRP (Properties)
(unclaimed protein sequence; signal peptides, nucleic acid mols. and methods for treatment of caries)

IT **438620-89-4**
RL: PRP (Properties)
(unclaimed sequence; signal peptides, nucleic acid mols. and methods for treatment of caries)

L2 ANSWER 2 OF 2 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2001:64716 HCAPLUS
DOCUMENT NUMBER: 134:277785
TITLE: Natural genetic transformation of Streptococcus mutans growing in biofilms
AUTHOR(S): Li, Yung-Hua; Lau, Peter C. Y.; Lee, Janet H.; Ellen, Richard P.; Cvitkovitch, Dennis G.
CORPORATE SOURCE: Dental Research Institute, University of Toronto, Toronto, ON, M5G 1G6, Can.
SOURCE: Journal of Bacteriology (2001), 183(3), 897-908
CODEN: JOBAAY; ISSN: 0021-9193
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Streptococcus mutans is a bacterium that has evolved to be dependent upon a biofilm "lifestyle" for survival and persistence in its natural ecosystem, dental plaque. We initiated this study to identify the genes involved in the development of genetic competence in S. mutans and to assay the natural genetic transformability of biofilm-grown cells. Using genomic analyses, we identified a quorum-sensing peptide pheromone signaling system similar to those previously found in other streptococci. The genetic locus of this system comprises three genes, comC, comD, and comE, that encode a precursor to the peptide competence factor, a histidine kinase, and a response regulator, resp. We deduced the sequence of comC and its active pheromone product and chem. synthesized the corresponding 21-amino-acid competence-stimulating peptide (CSP). Addn. of CSP to noncompetent cells facilitated increased transformation frequencies, with typically 1% of the total cell population transformed. To further confirm the roles of these genes in genetic competence, we inactivated them by insertion-duplication mutagenesis or allelic replacement followed by assays of transformation efficiency. We also demonstrated that biofilm-grown S. mutans cells were transformed at a rate 10- to 600-fold higher than planktonic S. mutans cells. Donor DNA included a suicide plasmid, S. mutans chromosomal DNA harboring a heterologous erythromycin resistance gene, and a replicative plasmid. The cells were optimally transformed during the formation of 8- to 16-h-old biofilms primarily consisting of microcolonies on solid surfaces. We also found that dead cells in the biofilms could act as donors of a chromosomally encoded antibiotic resistance determinant. This work demonstrated that a peptide pheromone system controls genetic competence in S. mutans and that the system functions optimally when the cells are living in actively growing biofilms.

IT **330647-51-3 330647-52-4**
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(amino acid sequence; natural genetic transformation of Streptococcus mutans growing in biofilms)

REFERENCE COUNT: 50 THERE ARE 50 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

(FILE 'HCAPLUS' ENTERED AT 14:51:25 ON 13 NOV 2002)
L3 5 SEA ABB=ON PLU=ON (CSP OR COMPETENC? SIGNAL PEPTIDE) AND ((STREPTOCOCC? OR S)(W)MUTANS)
L4 3 SEA ABB=ON PLU=ON L3 NOT L2

- key terms

Searcher : Shears 308-4994

L4 ANSWER 1 OF 3 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2002:335980 HCAPLUS
DOCUMENT NUMBER: 137:60040
TITLE: A quorum-sensing signaling system essential for genetic competence in **Streptococcus mutans** is involved in biofilm formation
AUTHOR(S): Li, Yung-Hua; Tang, Nan; Aspiras, Marcelo B.; Lau, Peter C. Y.; Lec, Janet H.; Ellen, Richard P.; Cvitkovitch, Dennis G.
CORPORATE SOURCE: Dental Research Institute, University of Toronto, Toronto, ON, M5G 1G6, Can.
SOURCE: Journal of Bacteriology (2002), 184(10), 2699-2708
CODEN: JOBAAY; ISSN: 0021-9193
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English
AB In a previous study, a quorum-sensing signaling system essential for genetic competence in **Streptococcus mutans** was identified, characterized, and found to function optimally in biofilms. Here, the authors demonstrate that this system also plays a role in the ability of **S. mutans** to initiate biofilm formation. To test this hypothesis, **S. mutans** wild-type strain NG8 and its knockout mutants defective in comC, comD, comE, and comX, as well as a comCDE deletion mutant, were assayed for their ability to initiate biofilm formation. The spatial distribution and architecture of the biofilms were examd. by SEM and confocal scanning laser microscopy. The results showed that inactivation of any of the individual genes under study resulted in the formation of an abnormal biofilm. The comC mutant, unable to produce or secrete a competence-stimulating peptide (**CSP**), formed biofilms with altered architecture, whereas the comD and comE mutants, which were defective in sensing and responding to the **CSP**, formed biofilms with reduced biomass. Exogenous addn. of the **CSP** and complementation with a plasmid contg. the wild-type comC gene into the cultures restored the wild-type biofilm architecture of comC mutants but showed no effect on the comD, comE, or comX mutant biofilms. The fact that biofilms formed by comC mutants differed from the comD, comE, and comX mutant biofilms suggested that multiple signal transduction pathways were affected by **CSP**. Addn. of synthetic **CSP** into the culture medium or introduction of the wild-type comC gene on a shuttle vector into the comCDE deletion mutant partially restored the wild-type biofilm architecture and further supported this idea. It is concluded that the quorum-sensing signaling system essential for genetic competence in **S. mutans** is important for the formation of biofilms by this gram-pos. organism.
REFERENCE COUNT: 42 THERE ARE 42 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L4 ANSWER 2 OF 3 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2001:880043 HCAPLUS
DOCUMENT NUMBER: 136:163932
TITLE: Cell density modulates acid adaptation in **Streptococcus mutans**:

implications for survival in biofilms

AUTHOR(S): Li, Yung-Hua; Hanna, Michael N.; Svensater, Gunnel; Ellen, Richard P.; Cvitkovitch, Dennis G.

CORPORATE SOURCE: Dental Research Institute, University of Toronto, Toronto, ON, M5G 1G6, Can.

SOURCE: Journal of Bacteriology (2001), 183(23), 6875-6884
CODEN: JOBAAY; ISSN: 0021-9193

PUBLISHER: American Society for Microbiology

DOCUMENT TYPE: Journal

LANGUAGE: English

AB **Streptococcus mutans** normally colonizes dental biofilms and is regularly exposed to continual cycles of acidic pH during ingestion of fermentable dietary carbohydrates. The ability of **S. mutans** to survive at low pH is an important virulence factor in the pathogenesis of dental caries. Despite a few studies of the acid adaptation mechanism of this organism, little work has focused on the acid tolerance of **S. mutans** growing in high-cell-d. biofilms. It is unknown whether biofilm growth mode or high cell d. affects acid adaptation by **S. mutans**. This study was initiated to examine the acid tolerance response (ATR) of **S. mutans** biofilm cells and to det. the effect of cell d. on the induction of acid adaptation. **S. mutans** BM71 cells were first grown in broth cultures to examine acid adaptation assocd. with growth phase, cell d., carbon starvation, and induction by culture filtrates. The cells were also grown in a chemostat-based biofilm fermentor for biofilm formation. Adaptation of biofilm cells to low pH was established in the chemostat by the acid generated from excess glucose metab., followed by a pH 3.5 acid shock for 3 h. Both biofilm and planktonic cells were removed to assay percentages of survival. The results showed that **S. mutans** BM71 exhibited a log-phase ATR induced by low pH and a stationary-phase acid resistance induced by carbon starvation. Cell d. was found to modulate acid adaptation in **S. mutans** log-phase cells, since pre-adapted cells at a higher cell d. or from a dense biofilm displayed significantly higher resistance to the killing pH than the cells at a lower cell d. The log-phase ATR could also be induced by a neutralized culture filtrate collected from a low-pH culture, suggesting that the culture filtrate contained an extracellular induction component(s) involved in acid adaptation in **S. mutans**. Heat or proteinase treatment abolished the induction by the culture filtrate. The results also showed that mutants defective in the comC, -D, or -E genes, which encode a quorum-sensing system essential for cell d.-dependent induction of genetic competence, had a diminished log-phase ATR. Addn. of synthetic competence-stimulating peptide (**CSP**) to the comC mutant restored the ATR. This study demonstrated that cell d. and biofilm growth mode modulated acid adaptation in **S. mutans**, suggesting that optimal development of acid adaptation in this organism involves both low pH induction and cell-cell communication.

REFERENCE COUNT: 60 THERE ARE 60 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L4 ANSWER 3 OF 3 HCAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 1997:722780 HCAPLUS
DOCUMENT NUMBER: 128:58014
TITLE: Natural competence in the genus Streptococcus: evidence that streptococci can change pherotype by interspecies recombinational exchanges
AUTHOR(S): Havarstein, Leiv Sigve; Hakenbeck, Regine; Gaustad, Peter
CORPORATE SOURCE: Laboratory of Microbial Gene Technology, Department of Biotechnological Sciences, Agricultural University of Norway, Aas, N-1432, Norway
SOURCE: Journal of Bacteriology (1997), 179(21), 6589-6594
CODEN: JOBAAY; ISSN: 0021-9193
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB To map the incidence of natural competence in the genus Streptococcus, we used PCR to screen a no. of streptococcal strains for the presence of the recently identified competence regulation operon, contg. the comC, -D, and -E genes. This approach established that the operon is present in strains belonging to the S. mitis (Abiotrophia adiacens) and S. anginosus groups, but it was not detected in the other strains examd. Competence is induced in S. pneumoniae and S. gordonii by strain-specific peptide pheromones, competence-stimulating peptides (**CSPs**). With its unique primary structure, each **CSP** represents a sep. pheromone type (pherotype), which is recognized by the signaling domain of the downstream histidine kinase, ComD. Thus, all bacteria induced to competence by a particular **CSP** belong to the same pherotype. In this study, we identified a no. of new pherotypes by sequencing the genes encoding the **CSP** and its receptor from different streptococcal species. We found that in several cases, these genes have a mosaic structure which must have arisen as the result of recombination between two distinct allelic variants. The obsd. mosaic blocks encompass the region encoding the **CSP** and the **CSP**-binding domain of the histidine kinase. Consequently, the recombination events have led to switches in pherotype for the strains involved. This suggests a novel mechanism for the adaptation of naturally competent streptococci to new environmental conditions.

L5 6 SEA ABB=ON PLU=ON (CSP OR COMPETENC?(1W)PEPTIDE) AND ((STREPTOCOCC? OR S)(W)MUTANS)
L6 1 SEA ABB=ON PLU=ON L5 NOT (L2 OR L4)

L6 ANSWER 1 OF 1 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2000:682148 HCAPLUS
DOCUMENT NUMBER: 134:15097
TITLE: Genetic transformation in **Streptococcus mutans** requires a peptide secretion-like apparatus
AUTHOR(S): Petersen, F. C.; Scheie, A. Aa.
CORPORATE SOURCE: Department of Oral Biology, Dental Faculty, University of Oslo, Blindern, N-0316, Norway
SOURCE: Oral Microbiology and Immunology (2000), 15(5), 329-334
CODEN: OMIMEE; ISSN: 0902-0055

PUBLISHER: Munksgaard International Publishers Ltd.
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Competence for genetic transformation in Streptococcus pneumoniae and Streptococcus gordonii involves the ComAB secretion app., which is thought to export the **competence**-stimulating **peptide**. Homologous secretory systems are also used for the export of certain bacteriocins and bacteriocin-like peptides. In this study, a similar secretory app. was found in the **Streptococcus mutans** genome, and its role in transformation was investigated. Gene inactivation resulted in a mutant deficient in transformability. We suggest that secretion of a peptide, possibly the **competence**-stimulating **peptide** itself, is involved in competence induction also in **S. mutans**.
REFERENCE COUNT: 41 THERE ARE 41 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

(FILE 'MEDLINE, BIOSIS, EMBASE, WPIDS, CONFSCI, SCISEARCH, JICST-EPLUS, JAPIO' ENTERED AT 14:54:21 ON 13 NOV 2002)
L7 21 S L5
L8 10 DUP REM L7 (11 DUPLICATES REMOVED)

L8 ANSWER 1 OF 10 MEDLINE DUPLICATE 1
ACCESSION NUMBER: 2002238690 MEDLINE
DOCUMENT NUMBER: 21972785 PubMed ID: 11976299
TITLE: A quorum-sensing signaling system essential for genetic competence in **Streptococcus mutans** is involved in biofilm formation.
AUTHOR: Li Yung-Hua; Tang Nan; Aspiras Marcelo B; Lau Peter C Y; Lee Janet H; Ellen Richard P; Cvitkovitch Dennis G
CORPORATE SOURCE: Dental Research Institute, University of Toronto, 124 Edward Street, Toronto, Ontario, Canada M5G 1G6.
CONTRACT NUMBER: DE 013230-02 (NIDCR)
SOURCE: JOURNAL OF BACTERIOLOGY, (2002 May) 184 (10) 2699-708.
Journal code: 2985120R. ISSN: 0021-9193.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200205
ENTRY DATE: Entered STN: 20020429
Last Updated on STN: 20020528
Entered Medline: 20020522
AB In a previous study, a quorum-sensing signaling system essential for genetic competence in **Streptococcus mutans** was identified, characterized, and found to function optimally in biofilms (Li et al., J. Bacteriol. 183:897-908, 2001). Here, we demonstrate that this system also plays a role in the ability of **S. mutans** to initiate biofilm formation. To test this hypothesis, **S. mutans** wild-type strain NG8 and its knockout mutants defective in comC, comD, comE, and comX, as well as a comCDE deletion mutant, were assayed for their ability to initiate biofilm formation. The spatial distribution and architecture of the biofilms were examined by scanning electron microscopy and confocal scanning laser microscopy. The results

showed that inactivation of any of the individual genes under study resulted in the formation of an abnormal biofilm. The comC mutant, unable to produce or secrete a **competence**-stimulating **peptide** (**CSP**), formed biofilms with altered architecture, whereas the comD and comE mutants, which were defective in sensing and responding to the **CSP**, formed biofilms with reduced biomass. Exogenous addition of the **CSP** and complementation with a plasmid containing the wild-type comC gene into the cultures restored the wild-type biofilm architecture of comC mutants but showed no effect on the comD, comE, or comX mutant biofilms. The fact that biofilms formed by comC mutants differed from the comD, comE, and comX mutant biofilms suggested that multiple signal transduction pathways were affected by **CSP**. Addition of synthetic **CSP** into the culture medium or introduction of the wild-type comC gene on a shuttle vector into the comCDE deletion mutant partially restored the wild-type biofilm architecture and further supported this idea. We conclude that the quorum-sensing signaling system essential for genetic competence in **S. mutans** is important for the formation of biofilms by this gram-positive organism.

L8 ANSWER 2 OF 10 MEDLINE
ACCESSION NUMBER: 2002003418 MEDLINE
DOCUMENT NUMBER: 21623570 PubMed ID: 11751845
TITLE: Identification of a protein that inactivates the **competence**-stimulating **peptide** of Streptococcus pneumoniae.
AUTHOR: Berge Mathieu; Langen Hanno; Claverys Jean-Pierre; Martin Bernard
CORPORATE SOURCE: Laboratoire de Microbiologie et Genetique Moleculaire, UMR 5100 CNRS-Universite Paul Sabatier, 31062 Toulouse Cedex, France.
SOURCE: JOURNAL OF BACTERIOLOGY, (2002 Jan) 184 (2) 610-3. Journal code: 2985120R. ISSN: 0021-9193.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200201
ENTRY DATE: Entered STN: 20020102
Last Updated on STN: 20020201
Entered Medline: 20020131

AB Competence for genetic transformation of Streptococcus pneumoniae is a transient physiological property inducible by a **competence**-stimulating **peptide** (**CSP**). A 68-kDa **CSP**-inactivating protein was previously obtained following lithium chloride (LiCl) extraction. By the same protocol, a **CSP**-inactivating protein was purified and identified by matrix-assisted laser desorption ionization-time of flight mass spectrometry as an endopeptidase, PepO. Analysis of a pepO mutant provided no support for the hypothesis that PepO participates in competence regulation. To reconcile in vitro and in vivo data, we suggest that LiCl treatment results in the release of intracellular molecules, including PepO.

L8 ANSWER 3 OF 10 WPIDS (C) 2002 THOMSON DERWENT
ACCESSION NUMBER: 2002-242173 [30] WPIDS
CROSS REFERENCE: 2002-242151 [30]

DOC. NO. NON-CPI: N2002-187203
DOC. NO. CPI: C2002-073080
TITLE: Novel compound that competitively inhibits binding of **competence** signal **peptide** to **Streptococcus mutans** histidine kinase, useful in treatment or prophylaxis of caries or endocarditis.
DERWENT CLASS: B04 C06 D16 D21 S03
INVENTOR(S): CVITKOVITCH, D G; LAU, P C; LI, Y H; CVITKOVITCH, D; LAU, P C Y
PATENT ASSIGNEE(S): (CVIT-I) CVITKOVITCH D G; (LAUP-I) LAU P C; (LIYH-I) LI Y H; (CVIT-I) CVITKOVITCH D; (LAUP-I) LAU P C Y
COUNTRY COUNT: 2
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
CA 2332733	A1	20011010	(200230)*	EN	82
US 2002081302	A1	20020627	(200245)		

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
CA 2332733	A1	CA 2001-2332733	20010220
US 2002081302	A1 Provisional	US 2001-269949P	20010220
		US 2001-833017	20010917

PRIORITY APPLN. INFO: CA 2000-2302861 20000410
AN 2002-242173 [30] WPIDS
CR 2002-242151 [30]
AB CA 2332733 A UPAB: 20020717
NOVELTY - A compound (I) that competitively inhibits binding of **competence** signal **peptide** (**CSP**) to **Streptococcus mutans** histidine kinase, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

(1) a pharmaceutical composition (II) comprising all or part of (I) which is preferably a peptide;

(2) an isolated nucleic acid molecule (III) encoding a **S.mutans CSP**, or a fragment of a peptide having **CSP** activity;

(3) a **CSP** nucleic acid molecule (IV) isolated from **S.mutans** or its fragment having **CSP** activity;

(4) a recombinant nucleic acid molecule (V) comprising (III) or (IV), and a constitutive promoter sequence or an inducible promoter sequence, operatively linked so that the promoter enhances transcription of the nucleic acid molecule in a host cell;

(5) a vector (VI) comprising (III) or (IV);

(6) a host cell (VII) comprising (V), (VI), or progeny of (VII);

(7) producing (M1) a recombinant host cell capable of expressing (III) or (IV), involves introducing (VI) into the host cell;

(8) expressing (M2) a peptide in a host cell produced by M1, by

culturing the host cell under conditions suitable for gene expression;

(9) an isolated polypeptide (VIII) encoded by and/or produced from (III), (IV), or (VI);

(10) an isolated **CSP** (IX) or its fragment having **S.mutans CSP** activity;

(11) a polypeptide fragment (X) of (IX) or a peptide mimetic of **CSP**;

(12) a polypeptide (XI) comprising a sequence having greater than 30%, 50% or 60% sequence identity to (IX);

(13) an isolated nucleic acid molecule (XII) encoding (VIII), (IX), (X) or (XI);

(14) an antibody (XIII) directed against (VIII), (IX), (X) or (XI);

(15) a vaccine composition (XIV) comprising all or part of (VIII), (IX), (X) or (XI); and

(16) evaluating (M3) caries-reducing properties of a compound involves:

(a) contacting the compound with (i) a **CSP**, a histidine kinase (HK)-binding fragment of **CSP**, or their derivatives, and (ii) HK, a **CSP** binding fragment of HK, or their derivatives, where (i) and (ii) are capable of binding, and determining the ability of the compound of interfere with the binding of (i) with (ii), where the ability to interfere with binding indicates that the compound reduces caries; or

(b) contacting the compound with a DNA vector encoding a marker gene, and a **S.mutans** culture, and determining whether the compound reduces uptake of the DNA vector into the **S.mutans** culture, where reduced uptake of the DNA vector indicates that the compound reduces caries.

ACTIVITY - Antibacterial. No biodata is given in the source material.

MECHANISM OF ACTION - Inhibitor of binding of **CSP** to **S.mutans** HK; vaccine (claimed); inhibitor of microbial biofilms involved in infections.

USE - (I) or (II) is useful in medical treatment or prophylaxis of caries or endocarditis (claimed). (I) is useful for inhibiting or disrupting microbial biofilms involved in infections in man and animals, and in biofouling of surfaces susceptible to microbial accumulation. (I) is useful for treatment or prophylaxis of a disease, disorder or abnormal physical state caused by **S. mutans**. (II) is useful for treating diseases caused by streptococcal infections. (III) is useful as probes or in assays to identify antagonists or inhibitors of **CSP** peptides. (VIII) is useful as an antigen for preparing (XIII), for in vitro analysis of HK, **CSP** or RR activity or structure, and in assays for the identification and developments of compounds to inhibit and/or enhance polypeptide or peptide function directly. (XIII) is useful for providing protection against caries, to screen organisms or tissues containing **CSP** peptide or **CSP**-like peptides, for immuno-purification of **CSP** or **CSP** -like peptides from crude extracts, and to detect **CSP** or a similar peptide.

Dwg.0/12

L8 ANSWER 4 OF 10 WPIDS (C) 2002 THOMSON DERWENT
ACCESSION NUMBER: 2002-242151 [30] WPIDS
CROSS REFERENCE: 2002-242173 [30]

DOC. NO. NON-CPI: N2002-187183
DOC. NO. CPI: C2002-073075
TITLE: Novel compound that inhibits binding of **competence** signal **peptide** of **Streptococcus mutans** to **S**. **mutans** histidine kinase, useful for treating or preventing caries or endocarditis.
DERWENT CLASS: B04 C06 D16 D21 S03
INVENTOR(S): CVITKOVITCH, D G; LAU, P C Y; LI, Y H; CVITKOVITCH, D
PATENT ASSIGNEE(S): (CVIT-I) CVITKOVITCH D G; (LAUP-I) LAU P C Y; (LIYH-I) LI Y H; (CVIT-I) CVITKOVITCH D
COUNTRY COUNT: 2
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
CA 2302861	A1	20011010	(200230)*	EN	49
US 2002081302	A1	20020627	(200245)		

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
CA 2302861	A1	CA 2000-2302861	20000410
US 2002081302	A1 Provisional	US 2001-269949P	20010220
		US 2001-833017	20010917

PRIORITY APPLN. INFO: CA 2000-2302861 20000410; CA 2001-2332733 20010220
AN 2002-242151 [30] WPIDS
CR 2002-242173 [30]
AB CA 2302861 A UPAB: 20020717
NOVELTY - A compound (I) that competitively inhibits binding of **competence** signal **peptide** (**CSP**) to **Streptococcus mutans** histidine kinase, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

(1) a pharmaceutical composition (II) comprising (I) and a carrier;

(2) an isolated nucleic acid (III) encoding **S**. **mutans CSP**, or a fragment of a peptide having **CSP** activity. (III) comprises a (a, b, c, d or e):

(a) nucleic acid molecule that hybridizes to all or part of a nucleic acid molecule having a fully defined sequence of AGCGGAAGCCTATCAACATTTTTCCGGCTGTTTAACAGAAGTTTTACACAAGCTTTGGGAAAA (S1), the fragment of (S1) encoding Ser Gly Ser Leu Ser Thr Phe Phe Arg Leu Phe Asn Arg Ser Phe Thr Gln Ala Leu Gly Lys (S2), or its complement under moderate or high stringency hybridization conditions;

(b) nucleic acid molecule which is degenerate with respect to (a);

(c) nucleic acid molecule of coding strand in (c) or its complement;

(d) nucleic acid molecule encoding the same amino acid sequence as (c); or

(e) nucleic acid molecule having at least 50% or 60% identity

with the nucleotide sequence of (c) or fragment of (S1) encoding (S2). The **CSP** nucleic acid molecule is isolated from **S. mutans**;

(3) a recombinant nucleic acid molecule (IV) comprising (III) and a constitutive promoter sequence or an inducible promoter sequence, operatively linked so that the promoter enhances transcription of nucleic acid molecule in host cell;

(4) a vector (V) comprising (III);

(5) a host cell (VI), or its progeny comprising (IV) or (V);

(6) an isolated polypeptide (P) encoded by and/or produced by (III) or (V);

(7) an isolated **CSP** (VII) or its fragments having **S. mutans CSP** activity;

(8) a polypeptide fragment (VIII) of (VII) having a sequence of (S2) or a peptide mimetic of the **CSP**;

(9) a polypeptide (IX) comprising a sequence having greater than 30%, 50% or 60% sequence identity to (VIII);

(10) an isolated nucleic acid molecule encoding (P), (VII)-(IX);

(11) an antibody (X) directed against (P), (VII)-(IX);

(12) vaccine composition (XI) comprising all or part of (P), (VII)-(IX); and

(13) evaluating caries-reducing properties of compound involves:

(a) contacting the compound with (i) **CSP**, a histidine kinase (HK)-binding fragment of **CSP** or their derivatives, and (ii) HK, a **CSP** binding fragment of HK or their derivatives, where (i) and (ii) are capable of binding; and

(b) determining the ability of the compound to interfere with the binding of (i) with (ii), where the ability to interfere with binding indicates that the compound reduces caries. Optionally, the method involves contacting the compound with a DNA vector encoding a marker gene, and **S. mutans** culture, and determining whether the compound reduces uptake of the DNA vector into the **S. mutans** culture, the reduced uptake of the DNA vector indicating that the compound reduces caries.

ACTIVITY - Antibacterial; antiinflammatory. No supporting data is given.

MECHANISM OF ACTION - Binding of **CSP** to **S. mutans** histidine kinase inhibitor; blocks signal molecule from activating histidine kinase receptor molecule; inhibits the stimulatory action of **CSP** on biofilm formation and acid tolerance of **S. mutans**; **CSP** inhibitor.

USE - (I) or (II) is useful for treating or prophylaxis of caries or endocarditis. (V) is useful for producing recombinant host cell capable of expressing (III). The recombinant host cell produced by the method is useful for expressing peptide in culture (all claimed). (III) is useful for identifying nucleic acid molecules encoding **CSP** activated peptide. (III) is also useful as probes and in assays to identify antagonists or inhibitors of the peptides produced by the nucleic acid molecules. (III) is also useful for preparing vaccines for preventing or treating the above mentioned conditions. Antibodies against **CSP** activity are also useful for preventing caries. The antibodies are also useful for screening organisms or tissues containing **CSP** peptide or **CSP**-like peptide, and for immunopurifying the peptides. The **CSP** nucleic acid molecules are useful in assays for genetic competence.

DESCRIPTION OF DRAWING(S) - The figure shows the arrangement of genetic locus encoding the signal peptide precursor (ComC), the histidine kinase (ComD) and the response regulator (ComE).
Dwg.1/1

L8 ANSWER 5 OF 10 MEDLINE DUPLICATE 2
ACCESSION NUMBER: 2001645969 MEDLINE
DOCUMENT NUMBER: 21555104 - PubMed ID: 11698377
TITLE: Cell density modulates acid adaptation in **Streptococcus mutans**: implications for survival in biofilms.
AUTHOR: Li Y H; Hanna M N; Svensater G; Ellen R P; Cvitkovitch D G
CORPORATE SOURCE: Dental Research Institute, University of Toronto, 124 Edward St., Toronto, Ontario M5G 1G6, Canada.
CONTRACT NUMBER: DE 013230-01 (NIDCR)
SOURCE: JOURNAL OF BACTERIOLOGY, (2001 Dec) 183 (23) 6875-84. Journal code: 2985120R. ISSN: 0021-9193.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200112
ENTRY DATE: Entered STN: 20011108
Last Updated on STN: 20020123
Entered Medline: 20011207

AB **Streptococcus mutans** normally colonizes dental biofilms and is regularly exposed to continual cycles of acidic pH during ingestion of fermentable dietary carbohydrates. The ability of **S. mutans** to survive at low pH is an important virulence factor in the pathogenesis of dental caries. Despite a few studies of the acid adaptation mechanism of this organism, little work has focused on the acid tolerance of **S. mutans** growing in high-cell-density biofilms. It is unknown whether biofilm growth mode or high cell density affects acid adaptation by **S. mutans**. This study was initiated to examine the acid tolerance response (ATR) of **S. mutans** biofilm cells and to determine the effect of cell density on the induction of acid adaptation. **S. mutans** BM71 cells were first grown in broth cultures to examine acid adaptation associated with growth phase, cell density, carbon starvation, and induction by culture filtrates. The cells were also grown in a chemostat-based biofilm fermentor for biofilm formation. Adaptation of biofilm cells to low pH was established in the chemostat by the acid generated from excess glucose metabolism, followed by a pH 3.5 acid shock for 3 h. Both biofilm and planktonic cells were removed to assay percentages of survival. The results showed that **S. mutans** BM71 exhibited a log-phase ATR induced by low pH and a stationary-phase acid resistance induced by carbon starvation. Cell density was found to modulate acid adaptation in **S. mutans** log-phase cells, since pre-adapted cells at a higher cell density or from a dense biofilm displayed significantly higher resistance to the killing pH than the cells at a lower cell density. The log-phase ATR could also be induced by a neutralized culture filtrate collected from a low-pH culture, suggesting that the culture filtrate contained an extracellular induction component(s) involved in acid adaptation in **S. mutans**. Heat or proteinase treatment abolished

the induction by the culture filtrate. The results also showed that mutants defective in the comC, -D, or -E genes, which encode a quorum sensing system essential for cell density-dependent induction of genetic competence, had a diminished log-phase ATR. Addition of synthetic **competence** stimulating **peptide** (**CSP**) to the comC mutant restored the ATR. This study demonstrated that cell density and biofilm growth mode modulated acid adaptation in **S. mutans**, suggesting that optimal development of acid adaptation in this organism involves both low pH induction and cell-cell communication.

L8 ANSWER 6 OF 10 MEDLINE DUPLICATE 3
ACCESSION NUMBER: 2001211701 MEDLINE
DOCUMENT NUMBER: 21142515 PubMed ID: 11208787
TITLE: Natural genetic transformation of **Streptococcus mutans** growing in biofilms.
AUTHOR: Li Y H; Lau P C; Lee J H; Ellen R P; Cvitkovitch D G
CORPORATE SOURCE: Dental Research Institute, University of Toronto, Toronto, Ontario, Canada M5G 1G6.
CONTRACT NUMBER: DE 013230-01 (NIDCR)
SOURCE: JOURNAL OF BACTERIOLOGY, (2001 Feb) 183 (3) 897-908. Journal code: 2985120R. ISSN: 0021-9193.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-AF277151; GENBANK-AF277157
ENTRY MONTH: 200104
ENTRY DATE: Entered STN: 20010425
Last Updated on STN: 20010425
Entered Medline: 20010419

AB **Streptococcus mutans** is a bacterium that has evolved to be dependent upon a biofilm "lifestyle" for survival and persistence in its natural ecosystem, dental plaque. We initiated this study to identify the genes involved in the development of genetic competence in **S. mutans** and to assay the natural genetic transformability of biofilm-grown cells. Using genomic analyses, we identified a quorum-sensing peptide pheromone signaling system similar to those previously found in other streptococci. The genetic locus of this system comprises three genes, comC, comD, and comE, that encode a precursor to the peptide competence factor, a histidine kinase, and a response regulator, respectively. We deduced the sequence of comC and its active pheromone product and chemically synthesized the corresponding 21-amino-acid **competence**-stimulating **peptide** (**CSP**). Addition of **CSP** to noncompetent cells facilitated increased transformation frequencies, with typically 1% of the total cell population transformed. To further confirm the roles of these genes in genetic competence, we inactivated them by insertion-duplication mutagenesis or allelic replacement followed by assays of transformation efficiency. We also demonstrated that biofilm-grown **S. mutans** cells were transformed at a rate 10- to 600-fold higher than planktonic **S. mutans** cells. Donor DNA included a suicide plasmid, **S. mutans** chromosomal DNA harboring a heterologous erythromycin resistance gene, and a replicative plasmid. The cells were optimally transformed during the formation of 8- to 16-h-old

biofilms primarily consisting of microcolonies on solid surfaces. We also found that dead cells in the biofilms could act as donors of a chromosomally encoded antibiotic resistance determinant. This work demonstrated that a peptide pheromone system controls genetic competence in **S. mutans** and that the system functions optimally when the cells are living in actively growing biofilms.

L8 ANSWER 7 OF 10 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 2002:212030 BIOSIS
DOCUMENT NUMBER: PREV200200212030
TITLE: A quorum-sensing system essential for induction of genetic competence in **Streptococcus mutans** is involved in biofilm formation.
AUTHOR(S): Li, Y. H. (1); Tang, N. (1); Chen, W. Y. (1); Cvitkovitch, D. G. (1)
CORPORATE SOURCE: (1) University of Toronto, Dental Research Institute, Toronto, ON Canada
SOURCE: Abstracts of the General Meeting of the American Society for Microbiology, (2001) Vol. 101, pp. 442. http://www.asmusa.org/mtgsrc/generalmeeting.htm. print.
Meeting Info.: 101st General Meeting of the American Society for Microbiology Orlando, FL, USA May 20-24, 2001
ISSN: 1060-2011.
DOCUMENT TYPE: Conference
LANGUAGE: English
AB In a previous study, we identified and characterized a cell-cell signaling system essential for genetic competence in **Streptococcus mutans**. This system consists of five gene products, a **competence**-stimulating **peptide** (**CSP**) encoded by comC, its dedicated secretion apparatus (ComAB), its histidine kinase receptor (ComD) and the cognate response regulator (ComE). We demonstrated that this quorum sensing system functioned optimally when the cells were living in actively growing biofilms, suggesting that this system might play a role in the development of **S. mutans** biofilms. To test this hypothesis, a wild-type **S. mutans** strain (NG8) and individual mutants defective in comAB, C, D, E and were assayed for their ability to form biofilm. Spatial distribution and architecture of biofilms were examined by scanning electron microscopy (SEM). Growth rates of the planktonically-grown cultures were also measured. The results showed that disruption of any of the genes under study resulted in a defect in biofilm formation. The comD and comE mutants had a two-fold decrease in biofilm mass when compared with the wild-type strain. The defect in biofilm formation by both mutants appeared to result from a decrease in their growth yields, although the resting cells of the comD mutant also showed a decrease in initial adherence to saliva- or mucin-coated polystyrene surfaces. Interestingly, the comAB and comC mutants showed a noticeable difference in biofilm architecture compared to the wild-type strain. Biofilms formed by these mutants appeared to be clumped together with 'web-like' micro-colonies. Addition of the synthetic **CSP** to growing cultures partially restored the wild-type biofilm structure. SEMs suggested that the variation in biofilm structure was likely due to formation of extremely long chains by these mutants, suggesting a link between the cell

signaling system and cell segregation during division. We conclude that the quorum-sensing signaling system essential for genetic competence in **S. mutans** is also involved in the formation of biofilms by this organism.

L8 ANSWER 8 OF 10 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 2002:211990 BIOSIS
DOCUMENT NUMBER: PREV200200211990
TITLE: Genetic transformation in **Streptococcus mutans**: Identification of competence genes by functional genomic analysis.
AUTHOR(S): Lee, J. H. (1); Lau, P. C. (1); Meloche, M. (1); Cutichia, J. (1); Ellen, R. P. (1); Cvitkovitch, D. G. (1)
CORPORATE SOURCE: (1) University of Toronto, Toronto, ON Canada
SOURCE: Abstracts of the General Meeting of the American Society for Microbiology, (2001) Vol. 101, pp. 432. http://www.asmusa.org/mtgsrc/generalmeeting.htm. print.
Meeting Info.: 101st General Meeting of the American Society for Microbiology Orlando, FL, USA May 20-24, 2001
ISSN: 1060-2011.
DOCUMENT TYPE: Conference
LANGUAGE: English
AB **Streptococcus mutans** enters a physiologic state called genetic competence for natural transformation to occur. We have previously described a peptide-mediated cell-cell signaling system involved in the induction of competence. As in other streptococci, the regulation of competence in **S. mutans** depends on a quorum-sensing system consisting of two genetic loci, comCDE and comAB. Genes in the locus comCDE respectively encode a **competence** stimulating **peptide** (**CSP**), its histidine kinase receptor and a response regulator. The competence locus, ComAB, encodes a **CSP** secretion apparatus. In this study, our goal was to identify other loci involved in competence by genomic analysis followed by phenotypic confirmation. Using tblastn, we searched the **S. mutans** genome database from the University of Oklahoma with known competence protein sequences of Streptococcus pneumoniae. We identified a gene encoding a putative global modulator, ComX, which links a quorum-sensing system to competence induction in S. pneumoniae, as well as competence operons homologous to comFA, cglABCDE, celAB, cinA-recA, and coiA whose gene products are believed to be involved in DNA uptake and recombination. The role of these genes in the genetic competence of **S. mutans** was confirmed by insertion-duplication mutagenesis or by the vectorless PCR-mediated mutagenesis. Mutants were assayed for transformation efficiency with and without the addition of synthetic **S. mutans CSP**. An examination of the kinetics of transformation, with the addition of the **CSP**, indicated that transient competence induction in **S. mutans** occurred at a specific cell density of O.D600apprx0.3 in mid-exponential phase. In summary, we demonstrated the presence and function of nine genes involved in the late competence phase of **S. mutans** using a combination of genomic analysis, mutagenesis, and physiologic tests of natural transformation.

L8 ANSWER 9 OF 10 MEDLINE
ACCESSION NUMBER: 2001477202 MEDLINE
DOCUMENT NUMBER: 21411738 PubMed ID: 11520610
TITLE: Different in vivo localization of the Escherichia coli proteins CspD and CspA.
COMMENT: Erratum in: FEMS Microbiol Lett 2002 Mar 5;208(2):305
AUTHOR: Giangrossi M; Exley R M; Le Hegarat F; Pon C L
CORPORATE SOURCE: Departimento di Biologia MCA, Universita di Camerino, I-62032, Camerino (MC), Italy.
SOURCE: FEMS MICROBIOLOGY LETTERS, (2001 Aug 21) 202 (2) 171-6.
Journal code: 7705721. ISSN: 0378-1097.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200110
ENTRY DATE: Entered STN: 20010827
Last Updated on STN: 20020821
Entered Medline: 20011025

AB Two **Csp** proteins (CspA and CspD) were fused to the green fluorescent protein GFP and expressed from their natural promoters or from an inducible promoter. Fluorescence microscopy and computerized image analysis indicate that in Escherichia coli growing at 37 degrees C CspD localizes in the nucleoid like the control H-NS while CspA occupies a polar position away from the nucleoid. Following cold shock CspA maintains its location, while CspD is not sufficiently expressed to permit its localization. The different localization of CspA and CspD indicates that these proteins play different roles in the cell in spite of their extensive structural similarity.

L8 ANSWER 10 OF 10 MEDLINE DUPLICATE 4
ACCESSION NUMBER: 2001118234 MEDLINE
DOCUMENT NUMBER: 21069963 PubMed ID: 11154426
TITLE: Genetic transformation in **Streptococcus mutans** requires a peptide secretion-like apparatus.
AUTHOR: Petersen F C; Scheie A A
CORPORATE SOURCE: Department of Oral Biology, Dental Faculty, University of Oslo, P.O. Box 1052 Blindern, N-0316 Oslo, Norway.
SOURCE: ORAL MICROBIOLOGY AND IMMUNOLOGY, (2000 Oct) 15 (5) 329-34.
Journal code: 8707451. ISSN: 0902-0055.
PUB. COUNTRY: Denmark
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Dental Journals
ENTRY MONTH: 200102
ENTRY DATE: Entered STN: 20010322
Last Updated on STN: 20010322
Entered Medline: 20010215

AB Competence for genetic transformation in Streptococcus pneumoniae and Streptococcus gordonii involves the ComAB secretion apparatus, which is thought to export the **competence**-stimulating **peptide**. Homologous secretory systems are also used for the export of certain bacteriocins and bacteriocin-like peptides. In

this study, a similar secretory apparatus was found in the **Streptococcus mutans** genome, and its role in transformation was investigated. Gene inactivation resulted in a mutant deficient in transformability. We suggest that secretion of a peptide, possibly the **competence**-stimulating **peptide** itself, is involved in competence induction also in **S. mutans.**

FILE 'USPATFULL' ENTERED AT 14:55:14 ON 13 NOV 2002
L9 4 S L5

L9 ANSWER 1 OF 4 USPATFULL
ACCESSION NUMBER: 2002:221971 USPATFULL
TITLE: ENTEROCOCCUS FAECALIS POLYNUCLEOTIDES AND POLYPEPTIDES
INVENTOR(S): KUNSCH, CHARLES A., ATLANTA, GA, UNITED STATES
DILLON, PATRICK J., CARLSBAD, CA, UNITED STATES
BARASH, STEVEN, ROCKVILLE, MD, UNITED STATES

	NUMBER	KIND	DATE	
PATENT INFORMATION:	US 2002120116	A1	20020829	
APPLICATION INFO.:	US 1998-70927	A1	19980504	(9)

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: HUMAN GENOME SCIENCES INC, 9410 KEY WEST AVENUE, ROCKVILLE, MD, 20850
NUMBER OF CLAIMS: 18
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 2 Drawing Page(s)
LINE COUNT: 13315
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention provides polynucleotide sequences of the genome of Enterococcus faecalis, polypeptide sequences encoded by the polynucleotide sequences, corresponding polynucleotides and polypeptides, vectors and hosts comprising the polynucleotides, and assays and other uses thereof. The present invention further provides polynucleotide and polypeptide sequence information stored on computer readable media, and computer-based systems and methods which facilitate its use.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
INCL INCLM: 536/023.200
INCLS: 435/069.100; 435/070.100; 435/071.100; 435/252.300; 435/320.100; 530/350.000; 530/387.900; 800/013.000
NCL NCLM: 536/023.200
NCLS: 435/069.100; 435/070.100; 435/071.100; 435/252.300; 435/320.100; 530/350.000; 530/387.900; 800/013.000

L9 ANSWER 2 OF 4 USPATFULL
ACCESSION NUMBER: 2002:156707 USPATFULL
TITLE: Signal peptides, nucleic acid molecules and methods for treatment of caries
INVENTOR(S): Cvitkovitch, Dennis, Oakville, CANADA
Lau, Peter C.Y., Richmond Hill, CANADA
Li, Yung Hua, Etobicoke, CANADA

NUMBER KIND DATE

Searcher : Shears 308-4994

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2002081302	A1	20020627
APPLICATION INFO.:	US 2001-833017	A1	20010917 (9)
PRIORITY INFORMATION:	CA 2000-2302861		20000410
	CA 2001-2332733		20010220
	US 2001-269949P		20010220 (60)

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: CONLEY ROSE & TAYON, P.C., P. O. BOX 3267, HOUSTON, TX, 77253-3267
NUMBER OF CLAIMS: 37
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 19 Drawing Page(s)
LINE COUNT: 2096
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The invention relates to a compound that competitively inhibits binding of **CSP** to **S. mutans** histidine kinase. The compound is preferably a peptide or an antibody. The compound is preferably a derivative of [SEQ ID NO:2], a fragment of [SEQ ID NO:2] or a derivative of a fragment of [SEQ ID NO:2].

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
INCL INCLM: 424/164.100
INCLS: 530/388.400; 536/023.700
NCL NCLM: 424/164.100
NCLS: 530/388.400; 536/023.700

L9 ANSWER 3 OF 4 USPATFULL
ACCESSION NUMBER: 2002:78228 USPATFULL
TITLE: IDENTIFICATION AND CHARACTERIZATION OF NOVEL PNEUMOCOCCAL CHOLINE BINDING PROTEIN, CBPG, AND DIAGNOSTIC AND THERAPEUTIC USES THEREOF
INVENTOR(S): TUOMANEN, ELAINE I., GERMANTOWN, TN, UNITED STATES
GOSINK, KHOOSHEH, CORDOVA, TN, UNITED STATES
MASURE, ROBERT, GERMANTOWN, TN, UNITED STATES

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2002041881	A1	20020411
APPLICATION INFO.:	US 1999-287070	A1	19990406 (9)
RELATED APPLN. INFO.:	Continuation-in-part of Ser. No. US 1998-196389, filed on 19 Nov 1998, ABANDONED		

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: DAVID A JACKSON ESQ, KLAUBER & JACKSON, 411 HACKENSACK AVENUE, HACKENSACK, NJ, 07601
NUMBER OF CLAIMS: 41
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 11 Drawing Page(s)
LINE COUNT: 2806
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention provides isolated polypeptides comprising an amino acid sequence of a choline binding protein CbpG. This

invention provides an isolated polypeptide comprising an amino acid sequence of a choline binding polypeptide CbpG or N-terminal CbpG truncate, including analogs, variants, mutants, derivatives and fragments thereof. This invention further provides an isolated immunogenic polypeptide comprising an amino acid sequence of a choline binding protein CbpG. This invention provides an isolated nucleic acid encoding a polypeptide comprising an amino acid sequence of a choline binding protein CbpG. This invention provides pharmaceutical compositions, vaccines, and diagnostic and therapeutic methods of use of the isolated polypeptides and nucleic acids. Assays for compounds which alter or inactivate the polypeptides of the present invention for use in therapy are also provided.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
INCL INCLM: 424/190.100
NCL NCLM: 424/190.100

L9 ANSWER 4 OF 4 USPATFULL
ACCESSION NUMBER: 2002:55159 USPATFULL
TITLE: STREPTOCOCCUS PNEUMONIAE POLYNUCLEOTIDES AND SEQUENCES
INVENTOR(S): KUNSCH, CHARLES A., GAITHERSBURG, MD, UNITED STATES
CHOI, GIL H., ROCKVILLE, MD, UNITED STATES
DILLON, PATRICK J., CARLSBAD, CA, UNITED STATES
ROSEN, CRAIG A., LAYTONSVILLE, MD, UNITED STATES
BARASH, STEVEN C., ROCKVILLE, MD, UNITED STATES
FANNON, MICHAEL R., SILVER SPRING, MD, UNITED STATES
DOUGHERTY, BRIAN A., MT. AIRY, MD, UNITED STATES

	NUMBER	KIND	DATE	
PATENT INFORMATION:	US 2002032323	A1	20020314	
	US 6420135	B2	20020716	
APPLICATION INFO.:	US 1997-961527	A1	19971030	(8)

	NUMBER	DATE
PRIORITY INFORMATION:	US 1996-29960P	19961031 (60)

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: HUMAN GENOME SCIENCES INC, 9410 KEY WEST AVENUE, ROCKVILLE, MD, 20850
NUMBER OF CLAIMS: 20
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 2 Drawing Page(s)
LINE COUNT: 7752
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention provides polynucleotide sequences of the genome of Streptococcus pneumoniae, polypeptide sequences encoded by the polynucleotide sequences, corresponding polynucleotides and polypeptides, vectors and hosts comprising the polynucleotides, and assays and other uses thereof. The present invention further provides polynucleotide and polypeptide sequence information stored on computer readable media, and computer-based systems and methods which facilitate its use.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
INCL INCLM: 536/023.700
INCLS: 536/024.320; 435/069.100; 435/320.100; 435/252.300
NCL NCLM: 435/069.100
NCLS: 435/252.300; 435/320.100; 435/325.000; 536/023.700

(FILE 'HCAPLUS, MEDLINE, BIOSIS, EMBASE, WPIDS, CONFSCI, SCISEARCH, JICST-EPLUS, JAPIO, USPATFULL' ENTERED AT 14:55:55 ON 13 NOV 2002)
L10 129 S "CVITKOVITCH D"?/AU
L11 1699 S "LAU P"?/AU
L12 62336 S "LI Y"?/AU
L13 17 S L10 AND L11 AND L12
L14 36 S L10 AND (L11 OR L12)
L15 17 S L11 AND L12
L16 32 S (L14 OR L10 OR L11 OR L12) AND (CSP OR COMPETEN?(1W)PEPTIDE)
L17 35 S L13 OR L15 OR L16
L18 16 DUP REM L17 (19 DUPLICATES REMOVED)

- Author(s)

L18 ANSWER 1 OF 16 HCAPLUS COPYRIGHT 2002 ACS DUPLICATE 1
ACCESSION NUMBER: 2002:488061 HCAPLUS
DOCUMENT NUMBER: 137:62148
TITLE: Signal peptides, nucleic acid molecules and methods for treatment of caries
INVENTOR(S): **Cvitkovitch, Dennis; Lau, Peter C. Y.; Li, Yung Hua**
PATENT ASSIGNEE(S): Can.
SOURCE: U.S. Pat. Appl. Publ., 50 pp.
CODEN: USXXCO
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 2002081302	A1	20020627	US 2001-833017	20010917
CA 2332733	AA	20011010	CA 2001-2332733	20010220

PRIORITY APPLN. INFO.: CA 2000-2302861 A 20000410
CA 2001-2332733 A 20010220
US 2001-269949P P 20010220

AB The invention relates to a compd. that competitively inhibits binding of **competence** signal **peptide** (**CSP**) to Streptococcus mutans histidine kinase. The compd. is preferably a peptide or an antibody. The compd. is preferably a deriv. of [SEQ ID NO:2], a fragment of [SEQ ID NO:2] or a deriv. of a fragment of [SEQ ID NO:2].

L18 ANSWER 2 OF 16 USPATFULL
ACCESSION NUMBER: 2002:109279 USPATFULL
TITLE: Electronic assembly with trench structures and methods of manufacture
INVENTOR(S): Figueroa, David G., Mesa, AZ, United States
Walk, Michael, Mesa, AZ, United States
Li, Yuan-Liang, Chandler, AZ, United States
Sankman, Robert L., Phoenix, AZ, United States
PATENT ASSIGNEE(S): Intel Corporation, Santa Clara, CA, United States

Searcher : Shears 308-4994

(U.S. corporation)

	NUMBER	KIND	DATE	
PATENT INFORMATION:	US 6388207	B1	20020514	
APPLICATION INFO.:	US 2000-751356		20001229	(9)
DOCUMENT TYPE:	Utility			
FILE SEGMENT:	GRANTED			
PRIMARY EXAMINER:	Paladini, Albert W.			
LEGAL REPRESENTATIVE:	Schwegman, Lundberg, Woessner & Kluth, P.A.			
NUMBER OF CLAIMS:	52			
EXEMPLARY CLAIM:	43			
NUMBER OF DRAWINGS:	25 Drawing Figure(s); 12 Drawing Page(s)			
LINE COUNT:	1003			

AB To accommodate the operational and structural requirements of high performance integrated circuits, an integrated circuit package includes conductive trenches that are formed within a substrate. The trenches provide increased current carrying capacity, lower inductance, higher capacitance, and single and/or dual reference planes for signal conductors. Trench structures can be provided at various locations within the substrate, such as adjacent to signal conductors and embedded capacitors, as well as on the substrate periphery to couple the package to a socket. Trenches can be formed by routing, drilling, imprinting, and/or microperforation. Methods of fabrication, as well as application of the package to an electronic assembly and to an electronic system, are also described.

L18 ANSWER 3 OF 16 HCAPLUS COPYRIGHT 2002 ACS DUPLICATE 2

ACCESSION NUMBER:	2002:842881 HCAPLUS
TITLE:	Novel two-component regulatory system involved in biofilm formation and acid resistance in Streptococcus mutans
AUTHOR(S):	**Li, Yung-Hua; Lau, Peter C. Y.** ; Tang, Nan; Svensater, Gunnel; Ellen, Richard P.; **Cvitkovitch, Dennis G.**
CORPORATE SOURCE:	Dental Research Institute, University of Toronto, Toronto, ON, M5G 1G6, Can.
SOURCE:	Journal of Bacteriology (2002), 184(22), 6333-6342 CODEN: JOBAAY; ISSN: 0021-9193
PUBLISHER:	American Society for Microbiology
DOCUMENT TYPE:	Journal
LANGUAGE:	English

AB The abilities of Streptococcus mutans to form biofilms and to survive acidic pH are regarded as two important virulence determinants in the pathogenesis of dental caries. Environmental stimuli are thought to regulate the expression of several genes assocd. with virulence factors through the activity of two-component signal transduction systems. Yet, little is known of the involvement of these systems in the physiol. and pathogenicity of S. mutans. In this study, we describe a two-component regulatory system and its involvement in biofilm formation and acid resistance in S. mutans. By searching the S. mutans genome database with tblastn with the HK03 and RR03 protein sequences from S. pneumoniae as queries, we identified two genes, designated hk11 and rr11, that encode a putative histidine kinase and its cognate response

regulator. To gain insight into their function, a PCR-mediated allelic-exchange mutagenesis strategy was used to create the hk11 (Emr) and rr11 (Emr) deletion mutants from S. mutans wild-type NG8 named SMHK11 and SMRR11, resp. The mutants were examd. for their growth rates, genetic competence, ability to form biofilms, and resistance to low-pH challenge. The results showed that deletion of hk11 or rr11 resulted in defects in biofilm formation and resistance to acidic pH. Both mutants formed biofilms with reduced biomass (50 to 70% of the d. of the parent strain). SEM revealed that the biofilms formed by the mutants had sponge-like architecture with what appeared to be large gaps that resembled water channel-like structures. The mutant biofilms were composed of longer chains of cells than those of the parent biofilm. Deletion of hk11 also resulted in greatly diminished resistance to low pH, although we did not observe the same effect when rr11 was deleted. Genetic competence was not affected in either mutant. The results suggested that the gene product of hk11 in S. mutans might act as a pH sensor that could cross talk with one or more response regulators. We conclude that the two-component signal transduction system encoded by hk11 and rr11 represents a new regulatory system involved in biofilm formation and acid resistance in S. mutans.

L18 ANSWER 4 OF 16 HCAPLUS COPYRIGHT 2002 ACS DUPLICATE 3
ACCESSION NUMBER: 2002:335980 HCAPLUS
DOCUMENT NUMBER: 137:60040
TITLE: A quorum-sensing signaling system essential for genetic competence in Streptococcus mutans is involved in biofilm formation
AUTHOR(S): **Li, Yung-Hua**; Tang, Nan; Aspiras, Marcelo B.; **Lau, Peter C. Y.**; Lec, Janet H.; Ellen, Richard P.; **Cvitkovitch, Dennis G.**
CORPORATE SOURCE: Dental Research Institute, University of Toronto, Toronto, ON, M5G 1G6, Can.
SOURCE: Journal of Bacteriology (2002), 184(10), 2699-2708
CODEN: JOBAAY; ISSN: 0021-9193
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English
AB In a previous study, a quorum-sensing signaling system essential for genetic competence in Streptococcus mutans was identified, characterized, and found to function optimally in biofilms. Here, the authors demonstrate that this system also plays a role in the ability of S. mutans to initiate biofilm formation. To test this hypothesis, S. mutans wild-type strain NG8 and its knockout mutants defective in comC, comD, comE, and comX, as well as a comCDE deletion mutant, were assayed for their ability to initiate biofilm formation. The spatial distribution and architecture of the biofilms were examd. by SEM and confocal scanning laser microscopy. The results showed that inactivation of any of the individual genes under study resulted in the formation of an abnormal biofilm. The comC mutant, unable to produce or secrete a **competence** -stimulating **peptide** (**CSP**), formed biofilms with altered architecture, whereas the comD and comE mutants, which were defective in sensing and responding to the **CSP**, formed biofilms with reduced biomass. Exogenous addn. of the **CSP** and complementation with a plasmid contg. the wild-type comC gene

into the cultures restored the wild-type biofilm architecture of comC mutants but showed no effect on the comD, comE, or comX mutant biofilms. The fact that biofilms formed by comC mutants differed from the comD, comE, and comX mutant biofilms suggested that multiple signal transduction pathways were affected by **CSP** . Addn. of synthetic **CSP** into the culture medium or introduction of the wild-type comC gene on a shuttle vector into the comCDE deletion mutant partially restored the wild-type biofilm architecture and further supported this idea. It is concluded that the quorum-sensing signaling system essential for genetic competence in S. mutans is important for the formation of biofilms by this gram-pos. organism.

REFERENCE COUNT: 42 THERE ARE 42 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L18 ANSWER 5 OF 16 SCISEARCH COPYRIGHT 2002 ISI (R)
ACCESSION NUMBER: 2002:596470 SCISEARCH
THE GENUINE ARTICLE: 559KE
TITLE: A novel two-component regulatory system involved in biofilm formation and acid resistance in streptococcus mutans.
AUTHOR: **Li Y H (Reprint)**; **Lau P C Y**; Tang N; **Cvitkovitch D G**
CORPORATE SOURCE: Univ Toronto, Toronto, ON, Canada
COUNTRY OF AUTHOR: Canada
SOURCE: JOURNAL OF DENTAL RESEARCH, (MAR 2002) Vol. 81, Sp. iss. SI, pp. A444-A444. MA 3617.
Publisher: INT AMER ASSOC DENTAL RESEARCHI A D R/A A D R, 1619 DUKE ST, ALEXANDRIA, VA 22314-3406 USA.
ISSN: 0022-0345.
DOCUMENT TYPE: Conference; Journal
LANGUAGE: English
REFERENCE COUNT: 0

L18 ANSWER 6 OF 16 HCAPLUS COPYRIGHT 2002 ACS DUPLICATE 4
ACCESSION NUMBER: 2002:795142 HCAPLUS
TITLE: Enantioseparation of novel chiral tetrahedrane-type clusters on an amylose tris(phenylcarbamate) chiral stationary phase
AUTHOR(S): Han, Xiaoqian; Liu, Yueqi; Zhang, Yuhua; Zhang, Weiqiang; **Li, Yongmin**; Chen, Liren
CORPORATE SOURCE: Lanzhou Institute of Chemical Physics, Chinese Academy of Sciences, Lanzhou, 730000, Peop. Rep. China
SOURCE: Chromatographia (2002), 56(5/6), 319-322
CODEN: CHRGB7; ISSN: 0009-5893
PUBLISHER: Friedrich Vieweg & Sohn Verlagsgesellschaft mbH
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Amylose tris(phenylcarbamate)(ATPC) coated on a small particle silica gel was prepd. This ATPC chiral stationary phase (ATPC-**CSP**) was found to be useful for the enantiomeric sepn. of some novel chiral tetrahedrane-type clusters. Moreover, the influence of mobile phase modifier and of the structure of chiral tetrahedrane-type clusters on the chiral sepn. and retention were investigated. The results suggest that not only the structure and concn. of alc. in mobile phase, but also the subtle structural

differences in racemates can have a pronounced effect on enantiomeric sepn. and retention.

REFERENCE COUNT: 9 THERE ARE 9 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L18 ANSWER 7 OF 16 WPIDS (C) 2002 THOMSON DERWENT
ACCESSION NUMBER: 2002-242173 [30] WPIDS
CROSS REFERENCE: 2002-242151 [30]
DOC. NO. NON-CPI: N2002-187203
DOC. NO. CPI: C2002-073080
TITLE: Novel compound that competitively inhibits binding of **competence** signal **peptide** to Streptococcus mutans histidine kinase, useful in treatment or prophylaxis of caries or endocarditis.
DERWENT CLASS: B04 C06 D16 D21 S03
INVENTOR(S): **CVITKOVITCH, D G; LAU, P C; LI, Y H; CVITKOVITCH, D; LAU, P C Y**
PATENT ASSIGNEE(S): (CVIT-I) CVITKOVITCH D G; (LAUP-I) LAU P C; (LIYH-I) LI Y H; (CVIT-I) CVITKOVITCH D; (LAUP-I) LAU P C Y
COUNTRY COUNT: 2
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
CA 2332733	A1	20011010	(200230)*	EN	82
US 2002081302	A1	20020627	(200245)		

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
CA 2332733	A1	CA 2001-2332733	20010220
US 2002081302	A1 Provisional	US 2001-269949P	20010220
		US 2001-833017	20010917

PRIORITY APPLN. INFO: CA 2000-2302861 20000410
AN 2002-242173 [30] WPIDS
CR 2002-242151 [30]
AB CA 2332733 A UPAB: 20020717
NOVELTY - A compound (I) that competitively inhibits binding of **competence** signal **peptide** (**CSP**) to Streptococcus mutans histidine kinase, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

(1) a pharmaceutical composition (II) comprising all or part of (I) which is preferably a peptide;

(2) an isolated nucleic acid molecule (III) encoding a S.mutans **CSP**, or a fragment of a peptide having **CSP** activity;

(3) a **CSP** nucleic acid molecule (IV) isolated from S.mutans or its fragment having **CSP** activity;

(4) a recombinant nucleic acid molecule (V) comprising (III) or (IV), and a constitutive promoter sequence or an inducible promoter sequence, operatively linked so that the promoter enhances

transcription of the nucleic acid molecule in a host cell;

(5) a vector (VI) comprising (III) or (IV);

(6) a host cell (VII) comprising (V), (VI), or progeny of (VII);

(7) producing (M1) a recombinant host cell capable of expressing (III) or (IV), involves introducing (VI) into the host cell;

(8) expressing (M2) a peptide in a host cell produced by M1, by culturing the host cell under conditions suitable for gene expression;

(9) an isolated polypeptide (VIII) encoded by and/or produced from (III), (IV), or (VI);

(10) an isolated **CSP** (IX) or its fragment having S.mutans **CSP** activity;

(11) a polypeptide fragment (X) of (IX) or a peptide mimetic of **CSP**;

(12) a polypeptide (XI) comprising a sequence having greater than 30%, 50% or 60% sequence identity to (IX);

(13) an isolated nucleic acid molecule (XII) encoding (VIII), (IX), (X) or (XI);

(14) an antibody (XIII) directed against (VIII), (IX), (X) or (XI);

(15) a vaccine composition (XIV) comprising all or part of (VIII), (IX), (X) or (XI); and

(16) evaluating (M3) caries-reducing properties of a compound involves:

(a) contacting the compound with (i) a **CSP**, a histidine kinase (HK)-binding fragment of **CSP**, or their derivatives, and (ii) HK, a **CSP** binding fragment of HK, or their derivatives, where (i) and (ii) are capable of binding, and determining the ability of the compound of interfere with the binding of (i) with (ii), where the ability to interfere with binding indicates that the compound reduces caries; or

(b) contacting the compound with a DNA vector encoding a marker gene, and a S.mutans culture, and determining whether the compound reduces uptake of the DNA vector into the S.mutans culture, where reduced uptake of the DNA vector indicates that the compound reduces caries.

ACTIVITY - Antibacterial. No biodata is given in the source material.

MECHANISM OF ACTION - Inhibitor of binding of **CSP** to S.mutans HK; vaccine (claimed); inhibitor of microbial biofilms involved in infections.

USE - (I) or (II) is useful in medical treatment or prophylaxis of caries or endocarditis (claimed). (I) is useful for inhibiting or disrupting microbial biofilms involved in infections in man and animals, and in biofouling of surfaces susceptible to microbial accumulation. (I) is useful for treatment or prophylaxis of a disease, disorder or abnormal physical state caused by S.mutans. (II) is useful for treating diseases caused by streptococcal infections. (III) is useful as probes or in assays to identify antagonists or inhibitors of **CSP** peptides. (VIII) is useful as an antigen for preparing (XIII), for in vitro analysis of HK, **CSP** or RR activity or structure, and in assays for the identification and developments of compounds to inhibit and/or enhance polypeptide or peptide function directly. (XIII) is useful for providing protection against caries, to screen organisms or tissues containing **CSP** peptide or **CSP**-like

peptides, for immuno-purification of **CSP** or **CSP** -like peptides from crude extracts, and to detect **CSP** or a similar peptide.
Dwg.0/12

L18 ANSWER 8 OF 16 WPIDS (C) 2002 THOMSON DERWENT
ACCESSION NUMBER: 2002-242151 [30] WPIDS
CROSS REFERENCE: 2002-242173 [30]
DOC. NO. NON-CPI: N2002-187183
DOC. NO. CPI: C2002-073075
TITLE: Novel compound that inhibits binding of **competence** signal **peptide** of Streptococcus mutans to S. mutans histidine kinase, useful for treating or preventing caries or endocarditis.
DERWENT CLASS: B04 C06 D16 D21 S03
INVENTOR(S): **CVITKOVITCH, D G; LAU, P C Y; LI, Y H; CVITKOVITCH, D**
PATENT ASSIGNEE(S): (CVIT-I) CVITKOVITCH D G; (LAUP-I) LAU P C Y; (LIYH-I) LI Y H; (CVIT-I) CVITKOVITCH D
COUNTRY COUNT: 2
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
CA 2302861	A1	20011010	(200230)*	EN	49
US 2002081302	A1	20020627	(200245)		

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
CA 2302861	A1	CA 2000-2302861	20000410
US 2002081302	A1 Provisional	US 2001-269949P	20010220
		US 2001-833017	20010917

PRIORITY APPLN. INFO: CA 2000-2302861 20000410; CA 2001-2332733 20010220
AN 2002-242151 [30] WPIDS
CR 2002-242173 [30]
AB CA 2302861 A UPAB: 20020717
NOVELTY - A compound (I) that competitively inhibits binding of **competence** signal **peptide** (**CSP**) to Streptococcus mutans histidine kinase, is new.
DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:
(1) a pharmaceutical composition (II) comprising (I) and a carrier;
(2) an isolated nucleic acid (III) encoding S. mutans **CSP**, or a fragment of a peptide having **CSP** activity. (III) comprises a (a, b, c, d or e):
(a) nucleic acid molecule that hybridizes to all or part of a nucleic acid molecule having a fully defined sequence of AGCGGAAGCCTATCAACATTTTTCCGGCTGTTTAACAGAAGTTTTACACAAGCTTTGGGAAAA (S1), the fragment of (S1) encoding Ser Gly Ser Leu Ser Thr Phe Phe Arg Leu Phe Asn Arg Ser Phe Thr Gln Ala Leu Gly Lys (S2), or its complement under moderate or high stringency hybridization

conditions;

(b) nucleic acid molecule which is degenerate with respect to (a);

(c) nucleic acid molecule of coding strand in (c) or its complement;

(d) nucleic acid molecule encoding the same amino acid sequence as (c); or

(e) nucleic acid molecule having at least 50% or 60% identity with the nucleotide sequence of (c) or fragment of (S1) encoding (S2). The **CSP** nucleic acid molecule is isolated from S. mutans;

(3) a recombinant nucleic acid molecule (IV) comprising (III) and a constitutive promoter sequence or an inducible promoter sequence, operatively linked so that the promoter enhances transcription of nucleic acid molecule in host cell;

(4) a vector (V) comprising (III);

(5) a host cell (VI), or its progeny comprising (IV) or (V);

(6) an isolated polypeptide (P) encoded by and/or produced by (III) or (V);

(7) an isolated **CSP** (VII) or its fragments having S. mutans **CSP** activity;

(8) a polypeptide fragment (VIII) of (VII) having a sequence of (S2) or a peptide mimetic of the **CSP**;

(9) a polypeptide (IX) comprising a sequence having greater than 30%, 50% or 60% sequence identity to (VIII);

(10) an isolated nucleic acid molecule encoding (P), (VII)-(IX);

(11) an antibody (X) directed against (P), (VII)-(IX);

(12) vaccine composition (XI) comprising all or part of (P), (VII)-(IX); and

(13) evaluating caries-reducing properties of compound involves:

(a) contacting the compound with (i) **CSP**, a histidine kinase (HK)-binding fragment of **CSP** or their derivatives, and (ii) HK, a **CSP** binding fragment of HK or their derivatives, where (i) and (ii) are capable of binding; and

(b) determining the ability of the compound to interfere with the binding of (i) with (ii), where the ability to interfere with binding indicates that the compound reduces caries. Optionally, the method involves contacting the compound with a DNA vector encoding a marker gene, and S. mutans culture, and determining whether the compound reduces uptake of the DNA vector into the S. mutans culture, the reduced uptake of the DNA vector indicating that the compound reduces caries.

ACTIVITY - Antibacterial; antiinflammatory. No supporting data is given.

MECHANISM OF ACTION - Binding of **CSP** to S. mutans histidine kinase inhibitor; blocks signal molecule from activating histidine kinase receptor molecule; inhibits the stimulatory action of **CSP** on biofilm formation and acid tolerance of S. mutans; **CSP** inhibitor.

USE - (I) or (II) is useful for treating or prophylaxis of caries or endocarditis. (V) is useful for producing recombinant host cell capable of expressing (III). The recombinant host cell produced by the method is useful for expressing peptide in culture (all claimed). (III) is useful for identifying nucleic acid molecules encoding **CSP** activated peptide. (III) is also useful as probes and in assays to identify antagonists or inhibitors of the

peptides produced by the nucleic acid molecules. (III) is also useful for preparing vaccines for preventing or treating the above mentioned conditions. Antibodies against **CSP** activity are also useful for preventing caries. The antibodies are also useful for screening organisms or tissues containing **CSP** peptide or **CSP**-like peptide, and for immunopurifying the peptides. The **CSP** nucleic acid molecules are useful in assays for genetic competence.

DESCRIPTION OF DRAWING(S) - The figure shows the arrangement of genetic locus encoding the signal peptide precursor (ComC), the histidine kinase (ComD) and the response regulator (ComE). Dwg.1/1

L18 ANSWER 9 OF 16 HCAPLUS COPYRIGHT 2002 ACS DUPLICATE 5

ACCESSION NUMBER: 2001:880043 HCAPLUS
DOCUMENT NUMBER: 136:163932
TITLE: Cell density modulates acid adaptation in Streptococcus mutans: implications for survival in biofilms
AUTHOR(S): **Li, Yung-Hua**; Hanna, Michael N.; Svensater, Gunnel; Ellen, Richard P.; **Cvitkovitch, Dennis G.**
CORPORATE SOURCE: Dental Research Institute, University of Toronto, Toronto, ON, M5G 1G6, Can.
SOURCE: Journal of Bacteriology (2001), 183(23), 6875-6884
CODEN: JOBAAY; ISSN: 0021-9193
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Streptococcus mutans normally colonizes dental biofilms and is regularly exposed to continual cycles of acidic pH during ingestion of fermentable dietary carbohydrates. The ability of S. mutans to survive at low pH is an important virulence factor in the pathogenesis of dental caries. Despite a few studies of the acid adaptation mechanism of this organism, little work has focused on the acid tolerance of S. mutans growing in high-cell-d. biofilms. It is unknown whether biofilm growth mode or high cell d. affects acid adaptation by S. mutans. This study was initiated to examine the acid tolerance response (ATR) of S. mutans biofilm cells and to det. the effect of cell d. on the induction of acid adaptation. S. mutans BM71 cells were first grown in broth cultures to examine acid adaptation assocd. with growth phase, cell d., carbon starvation, and induction by culture filtrates. The cells were also grown in a chemostat-based biofilm fermentor for biofilm formation. Adaptation of biofilm cells to low pH was established in the chemostat by the acid generated from excess glucose metab., followed by a pH 3.5 acid shock for 3 h. Both biofilm and planktonic cells were removed to assay percentages of survival. The results showed that S. mutans BM71 exhibited a log-phase ATR induced by low pH and a stationary-phase acid resistance induced by carbon starvation. Cell d. was found to modulate acid adaptation in S. mutans log-phase cells, since pre-adapted cells at a higher cell d. or from a dense biofilm displayed significantly higher resistance to the killing pH than the cells at a lower cell d. The log-phase ATR could also be induced by a neutralized culture filtrate collected from a low-pH culture, suggesting that the culture filtrate contained an extracellular induction component(s) involved in acid adaptation in

S. mutans. Heat or proteinase treatment abolished the induction by the culture filtrate. The results also showed that mutants defective in the comC, -D, or -E genes, which encode a quorum-sensing system essential for cell d.-dependent induction of genetic competence, had a diminished log-phase ATR. Addn. of synthetic **competence**-stimulating **peptide** (**CSP**) to the comC mutant restored the ATR. This study demonstrated that cell d. and biofilm growth mode modulated acid adaptation in S. mutans, suggesting that optimal development of acid adaptation in this organism involves both low pH induction and cell-cell communication.

REFERENCE COUNT: 60 THERE ARE 60 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L18 ANSWER 10 OF 16 HCAPLUS COPYRIGHT 2002 ACS DUPLICATE 6

ACCESSION NUMBER: 2001:64716 HCAPLUS
DOCUMENT NUMBER: 134:277785
TITLE: Natural genetic transformation of Streptococcus mutans growing in biofilms
AUTHOR(S): **Li, Yung-Hua; Lau, Peter C. Y.** ; Lee, Janet H.; Ellen, Richard P.; **Cvitkovitch, Dennis G.**
CORPORATE SOURCE: Dental Research Institute, University of Toronto, Toronto, ON, M5G 1G6, Can.
SOURCE: Journal of Bacteriology (2001), 183(3), 897-908 CODEN: JOBAAY; ISSN: 0021-9193
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Streptococcus mutans is a bacterium that has evolved to be dependent upon a biofilm "lifestyle" for survival and persistence in its natural ecosystem, dental plaque. We initiated this study to identify the genes involved in the development of genetic competence in S. mutans and to assay the natural genetic transformability of biofilm-grown cells. Using genomic analyses, we identified a quorum-sensing peptide pheromone signaling system similar to those previously found in other streptococci. The genetic locus of this system comprises three genes, comC, comD, and comE, that encode a precursor to the peptide competence factor, a histidine kinase, and a response regulator, resp. We deduced the sequence of comC and its active pheromone product and chem. synthesized the corresponding 21-amino-acid **competence**-stimulating **peptide** (**CSP**). Addn. of **CSP** to noncompetent cells facilitated increased transformation frequencies, with typically 1% of the total cell population transformed. To further confirm the roles of these genes in genetic competence, we inactivated them by insertion-duplication mutagenesis or allelic replacement followed by assays of transformation efficiency. We also demonstrated that biofilm-grown S. mutans cells were transformed at a rate 10- to 600-fold higher than planktonic S. mutans cells. Donor DNA included a suicide plasmid, S. mutans chromosomal DNA harboring a heterologous erythromycin resistance gene, and a replicative plasmid. The cells were optimally transformed during the formation of 8- to 16-h-old biofilms primarily consisting of microcolonies on solid surfaces. We also found that dead cells in the biofilms could act as donors of a chromosomally encoded antibiotic resistance determinant. This work demonstrated that a peptide pheromone system

controls genetic competence in S. mutans and that the system functions optimally when the cells are living in actively growing biofilms.

REFERENCE COUNT: 50 THERE ARE 50 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L18 ANSWER 11 OF 16 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 2002:212030 BIOSIS
DOCUMENT NUMBER: PREV200200212030
TITLE: A quorum-sensing system essential for induction of genetic competence in Streptococcus mutans is involved in biofilm formation.
AUTHOR(S): **Li, Y. H. (1)**; Tang, N. (1); Chen, W. Y. (1); **Cvitkovitch, D. G. (1)**
CORPORATE SOURCE: (1) University of Toronto, Dental Research Institute, Toronto, ON Canada
SOURCE: Abstracts of the General Meeting of the American Society for Microbiology, (2001) Vol. 101, pp. 442. http://www.asmusa.org/mtgsrc/generalmeeting.htm. print.
Meeting Info.: 101st General Meeting of the American Society for Microbiology Orlando, FL, USA May 20-24, 2001
ISSN: 1060-2011.
DOCUMENT TYPE: Conference
LANGUAGE: English

AB In a previous study, we identified and characterized a cell-cell signaling system essential for genetic competence in Streptococcus mutans. This system consists of five gene products, a **competence**-stimulating **peptide** (**CSP**) encoded by comC, its dedicated secretion apparatus (ComAB), its histidine kinase receptor (ComD) and the cognate response regulator (ComE). We demonstrated that this quorum sensing system functioned optimally when the cells were living in actively growing biofilms, suggesting that this system might play a role in the development of S. mutans biofilms. To test this hypothesis, a wild-type S. mutans strain (NG8) and individual mutants defective in comAB, C, D, E and were assayed for their ability to form biofilm. Spatial distribution and architecture of biofilms were examined by scanning electron microscopy (SEM). Growth rates of the planktonically-grown cultures were also measured. The results showed that disruption of any of the genes under study resulted in a defect in biofilm formation. The comD and comE mutants had a two-fold decrease in biofilm mass when compared with the wild-type strain. The defect in biofilm formation by both mutants appeared to result from a decrease in their growth yields, although the resting cells of the comD mutant also showed a decrease in initial adherence to saliva- or mucin-coated polystyrene surfaces. Interestingly, the comAB and comC mutants showed a noticeable difference in biofilm architecture compared to the wild-type strain. Biofilms formed by these mutants appeared to be clumped together with 'web-like' micro-colonies. Addition of the synthetic **CSP** to growing cultures partially restored the wild-type biofilm structure. SEMs suggested that the variation in biofilm structure was likely due to formation of extremely long chains by these mutants, suggesting a link between the cell signaling system and cell segregation during division. We conclude that the quorum-sensing signaling system essential for genetic

competence in S. mutans is also involved in the formation of biofilms by this organism.

L18 ANSWER 12 OF 16 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 2002:211990 BIOSIS
DOCUMENT NUMBER: PREV200200211990
TITLE: Genetic transformation in Streptococcus mutans: Identification of competence genes by functional genomic analysis.
AUTHOR(S): Lee, J. H. (1); **Lau, P. C. (1)**; Meloche, M. (1); Cutichia, J. (1); Ellen, R. P. (1); **Cvitkovitch, D. G. (1)**
CORPORATE SOURCE: (1) University of Toronto, Toronto, ON Canada
SOURCE: Abstracts of the General Meeting of the American Society for Microbiology, (2001) Vol. 101, pp. 432. http://www.asmusa.org/mtgsrc/generalmeeting.htm. print.
Meeting Info.: 101st General Meeting of the American Society for Microbiology Orlando, FL, USA May 20-24, 2001
ISSN: 1060-2011.
DOCUMENT TYPE: Conference
LANGUAGE: English
AB Streptococcus mutans enters a physiologic state called genetic competence for natural transformation to occur. We have previously described a peptide-mediated cell-cell signaling system involved in the induction of competence. As in other streptococci, the regulation of competence in S. mutans depends on a quorum-sensing system consisting of two genetic loci, comCDE and comAB. Genes in the locus comCDE respectively encode a **competence** stimulating **peptide** (**CSP**), its histidine kinase receptor and a response regulator. The competence locus, ComAB, encodes a **CSP** secretion apparatus. In this study, our goal was to identify other loci involved in competence by genomic analysis followed by phenotypic confirmation. Using tblastn, we searched the S. mutans genome database from the University of Oklahoma with known competence protein sequences of Streptococcus pneumoniae. We identified a gene encoding a putative global modulator, ComX, which links a quorum-sensing system to competence induction in S. pneumoniae, as well as competence operons homologous to comFA, cglABCDE, celAB, cinA-recA, and coiA whose gene products are believed to be involved in DNA uptake and recombination. The role of these genes in the genetic competence of S. mutans was confirmed by insertion-duplication mutagenesis or by the vectorless PCR-mediated mutagenesis. Mutants were assayed for transformation efficiency with and without the addition of synthetic S. mutans **CSP**. An examination of the kinetics of transformation, with the addition of the **CSP**, indicated that transient competence induction in S. mutans occurred at a specific cell density of O.D600apprx0.3 in mid-exponential phase. In summary, we demonstrated the presence and function of nine genes involved in the late competence phase of S. mutans using a combination of genomic analysis, mutagenesis, and physiologic tests of natural transformation.

L18 ANSWER 13 OF 16 HCAPLUS COPYRIGHT 2002 ACS DUPLICATE 7
ACCESSION NUMBER: 2001:97758 HCAPLUS
DOCUMENT NUMBER: 135:271437

TITLE: Assessment of a vaccinia virus vectored multi-epitope live vaccine candidate for Plasmodium falciparum
AUTHOR(S): Dong, W.; Li, M.; Bi, H.; **Li, Y.**; Wu, J.; Qu, L.
CORPORATE SOURCE: Institute of Tropical Medicine, First Military Medical University, Canton, 510515, Peop. Rep. China
SOURCE: International Journal for Parasitology (2001), 31(1), 57-62
CODEN: IJPYBT; ISSN: 0020-7519
PUBLISHER: Elsevier Science Ltd.
DOCUMENT TYPE: Journal
LANGUAGE: English

AB We constructed a live recombinant vaccinia virus vaccine candidate contg. a synthesized hybrid gene termed 'HGFSP' encoding circumsporozoite protein (**CSP**), major merozoite surface antigen-1(MSA1), major merozoite surface antigen-2 (MSA2), and ring-infected erythrocyte surface antigen (RESA) of Plasmodium falciparum, interleukin-1 (IL-1) and tetanus toxin (TT) epitopes. Anti-recombinant vaccinia virus rabbit sera and IgG were tested in inhibition expts. in vitro. Results showed that the recombinant vaccinia virus had some capability to inhibit the growth of P. falciparum in vitro. The sera of rabbits, rats, and mice immunized with recombinant virus showed obvious IL-2 activity 4-6 wk after immunization. The interferon (IFN) level of sera from these animals 6 wk after immunization was significantly higher than before immunization. These results indicate that the recombinant vaccinia virus can stimulate cell mediated responses (Th1 cell response) in immunized animals, and has the capability to inhibit multiplication of in vitro cultured P. falciparum. Thus this recombinant vaccinia virus is an appropriate vaccine candidate for further evaluation in Aotus monkey or human clin. trails.

REFERENCE COUNT: 22 THERE ARE 22 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L18 ANSWER 14 OF 16 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1999:506908 HCAPLUS
DOCUMENT NUMBER: 132:48689
TITLE: Construction of a vaccinia virus vectored multi-epitope live vaccine candidate for Plasmodium falciparum
AUTHOR(S): Dong, Wenqi; Li, Ming; Bi, Huixiang; **Li, Yingjie**
CORPORATE SOURCE: Institute of Tropical Medicine, First Military Medical University, Canton, 510515, Peop. Rep. China
SOURCE: Journal of Medical Colleges of PLA (1999), 14(2), 119-123
CODEN: JMCPE6; ISSN: 1000-1948
PUBLISHER: Journal of Medical Colleges of PLA, Editorial Board
DOCUMENT TYPE: Journal
LANGUAGE: English

AB To construct live recombinant vaccinia virus, the HGFSP gene encoding **CSP**, MSA1, MSA2, RESA, IL-1 and TT epitopes was inserted into the Eco RI and Bam HI sites of pSK plasmid. After

digestion with Eco RI, bluntly ended by Klenow enzyme and digested with Sac I, the HGFSP gene was cloned into the Sma I and Sac I sites of the vaccinia virus insertion vector (pJ2-16). Recombinant plasmids were identified by gel electrophoresis, restriction enzyme and enzyme map. Results evidenced that HGFSP gene fragment was correctly inserted into the cloning site of hemagglutinin (HA) gene of the pJ2-16 vector. The recombinant plasmids were transfected into Cos-7 cells, which were infected with wild type of vaccinia virus Tiantan strain, by means of lipofectamine. Two recombinant vaccinia viruses (HA-) were screened and cloned by chicken hemadsorption test in BHK21 cells. Indirect immunofluorescence assay (IFA), Dot-ELISA and Western blot with the antibodies against HGFSP protein expressed by E. coli showed that one of the 2 recombinant vaccinia virus expressed desired proteins in infected BHK21 cells. Western blot also showed that the mol. wt. of 2 expressed protein bands was about 23 kDa, according to the theor. mol. wt. of HGFSP protein. Further identification of immunol. characters of recombinant virus is under way.

REFERENCE COUNT: 9 THERE ARE 9 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L18 ANSWER 15 OF 16 MEDLINE
ACCESSION NUMBER: 2002336598 IN-PROCESS
DOCUMENT NUMBER: 22074050 PubMed ID: 12078270
TITLE: Cloning and sequence analysis of the gene encoding the partial region CS protein of a Plasmodium falciparum isolate from Yunnan.
AUTHOR: Xiao J; Li M; Chui D; Bi H; Wang P; **Li Y**
CORPORATE SOURCE: Institute of Tropical Medicine, First Military Medical University, Guangzhou 510515.
SOURCE: CHUNG-KUO CHI SHENG CHUNG HSUEH YU CHI SHENG CHUNG PING TSA CHIH CHINESE JOURNAL OF PARASITOLOGY AND PARASITIC DISEASES, (1998) 16 (5) 342-6.
Journal code: 8709992. ISSN: 1000-7423.
PUB. COUNTRY: China
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: Chinese
FILE SEGMENT: IN-PROCESS; NONINDEXED; Priority Journals
ENTRY DATE: Entered STN: 20020625
Last Updated on STN: 20020625

AB AIM: Determining nucleotide sequence of the circumsporozoite protein partial gene of the Plasmodium falciparum PFD-3/YN (Yunnan of China) and finding out the differences of the CS gene sequence between Chinese Plasmodium falciparum isolate and other isolates. METHODS: The circumsporozoite protein gene fragment was amplified by polymerase chain reaction and cloned into M13 bacteriophage. M13-**CSP** single strand DNAs of the three positive clones were extracted respectively. Then, the nucleotide sequence of the CS gene ragment was determined by the dideoxy chain termination method. PCGENE software was used to compare and analyze the CS gene sequence of the six isolates. RESULTS: Different degrees of diversity of the CS gene sequences were found among P. falciparum PFD-3/YN and other isolates(T4, Wellcome, NF54, 3D7 and 7G8). A non-silent substitution at the nucleotide level being found in the P. f Th/Tc antigenic epitope region. CONCLUSION: There were differences in the CS gene sequence among P. falciparum PFD-3/YN and those of other isolates.

L18 ANSWER 16 OF 16 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1998:287967 HCAPLUS
DOCUMENT NUMBER: 128:316731
TITLE: Study on enantioselectivity of chiral stationary phase-permethyl-2,3,6-tri(O-2'-racemic hydroxypropyl)-.beta.-cyclodextrin
AUTHOR(S): Ding, Yuqiang; **Li, Yan**; Zeng, Zhaorui; Wu, Caiying
CORPORATE SOURCE: Dep. Chem., Wuhan Univ., Wuhan, 430074, Peop. Rep. China
SOURCE: Sepu (1998), 16(2), 152-154
CODEN: SEPUER; ISSN: 1000-8713
PUBLISHER: Sepu Jishu Yanjiu Kaifa Zhongxin
DOCUMENT TYPE: Journal
LANGUAGE: Chinese
AB A highly selective chiral stationary phase (**CSP**), permethyl-2,3,6-tri(O-2'-RS-hydroxypropyl)-.beta.-cyclodextrin (PMRHP-.beta.-CD) was synthesized by using racemic propylene oxide and characterized by TLC, IR and NMR. The PMRHP-.beta.-CD was coated on a fused silica capillary column (0.25 mm .times. 16 m). The enantiomers, including alcs., an amino acid, a bromoalkane and an ester were used to test its enantioselectivity. The exptl. results show the high selectivity of this cyclodextrin deriv.

FILE 'HCAPLUS' ENTERED AT 14:59:13 ON 13 NOV 2002
L19 147 S COMPETEN?(1W)PROTEIN
L20 0 S L19 AND MUTANS

- Key terms

FILE 'MEDLINE, BIOSIS, EMBASE, WPIDS, CONFSCI, SCISEARCH, JICST-EPLUS, JAPIO' ENTERED AT 15:00:00 ON 13 NOV 2002
L21 1 S L20
L22 0 S L21 NOT L7

FILE 'USPATFULL' ENTERED AT 15:00:55 ON 13 NOV 2002
L23 5 S L20
L24 2 S L23 NOT L9

L24 ANSWER 1 OF 2 USPATFULL
ACCESSION NUMBER: 2002:297296 USPATFULL
TITLE: Methods for inhibition of membrane fusion-associated events, including respiratory syncytial virus transmission
INVENTOR(S): Bolognesi, Dani Paul, Durham, NC, United States
Matthews, Thomas James, Durham, NC, United States
Wild, Carl T., Durham, NC, United States
Barney, Shawn O'Lin, Cary, NC, United States
Lambert, Dennis Michael, Cary, NC, United States
Petteway, Stephen Robert, Cary, NC, United States
Langlois, Alphonse J., Durham, NC, United States
PATENT ASSIGNEE(S): Trimeris, Inc., Durham, NC, United States (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 6479055	B1	20021112
APPLICATION INFO.:	US 1995-470896		19950606 (8)

RELATED APPLN. INFO.: Continuation-in-part of Ser. No. US 1994-360107, filed on 20 Dec 1994, now patented, Pat. No. US

Searcher : Shears 308-4994

6017536 Continuation-in-part of Ser. No. US 1994-255208, filed on 7 Jun 1994 Continuation-in-part of Ser. No. US 1993-73028, filed on 7 Jun 1993, now patented, Pat. No. US 5464933
DOCUMENT TYPE: Utility
FILE SEGMENT: GRANTED
PRIMARY EXAMINER: Stucker, Jeffrey
LEGAL REPRESENTATIVE: Pennie & Edmonds LLP
NUMBER OF CLAIMS: 44
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 84 Drawing Figure(s); 83 Drawing Page(s)
LINE COUNT: 26553
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention relates to peptides which exhibit potent anti-viral activity. In particular, the invention relates to methods of using such peptides as inhibitory of respiratory syncytial virus ("RSV") transmission to uninfected cells. The peptides used in the methods of the invention are homologs of the DP-178 and DP-107 peptides, peptides corresponding to amino acid residues 638 to 673, and to amino acid residues 558 to 595, respectively, of the HIV-1.sub.LAI transmembrane protein (TM) gp41.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
INCL INCLM: 424/211.100
INCLS: 424/186.100; 530/324.000
NCL NCLM: 424/211.100
NCLS: 424/186.100; 530/324.000

L24 ANSWER 2 OF 2 USPATFULL
ACCESSION NUMBER: 2001:67794 USPATFULL
TITLE: Human respiratory syncytial virus peptides with antifusogenic and antiviral activities
INVENTOR(S): Barney, Shawn O'Lin, Cary, NC, United States
Lambert, Dennis Michael, Cary, NC, United States
Petteway, Stephen Robert, Cary, NC, United States
PATENT ASSIGNEE(S): Trimeris, Inc., Durham, NC, United States (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 6228983	B1	20010508
APPLICATION INFO.:	US 1995-485264		19950607 (8)

RELATED APPLN. INFO.: Division of Ser. No. US 1995-470896, filed on 6 Jun 1995 Continuation-in-part of Ser. No. US 1994-360107, filed on 20 Dec 1994 Continuation-in-part of Ser. No. US 1994-255208, filed on 7 Jun 1994 Continuation-in-part of Ser. No. US 1993-73028, filed on 7 Jun 1993, now patented, Pat. No. US 5464933
DOCUMENT TYPE: Utility
FILE SEGMENT: Granted
PRIMARY EXAMINER: Scheiner, Laurie
ASSISTANT EXAMINER: Parkin, Jeffrey S.
LEGAL REPRESENTATIVE: Pennie & Edmonds LLP
NUMBER OF CLAIMS: 62
EXEMPLARY CLAIM: 1

Searcher : Shears 308-4994

NUMBER OF DRAWINGS: 84 Drawing Figure(s); 83 Drawing Page(s)
LINE COUNT: 32166
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention relates to peptides which exhibit antifusogenic and antiviral activities. The peptides of the invention consist of a 16 to 39 amino acid region of a human respiratory syncytial virus protein. These regions were identified through computer algorithms capable of recognizing the ALLMOTI5, 107x178x4, or PLZIP amino acid motifs. These motifs are associated with the antifusogenic and antiviral activities of the claimed peptides.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
INCL INCLM: 530/300.000
INCLS: 530/324.000; 530/325.000; 530/326.000; 424/211.100; 424/186.100
NCL NCLM: 530/300.000
NCLS: 424/186.100; 424/211.100; 530/324.000; 530/325.000; 530/326.000

(FILE 'HCAPLUS, MEDLINE, BIOSIS, EMBASE, WPIDS, CONFSCI, SCISEARCH, JICST-EPLUS, JAPIO, USPATFULL' ENTERED AT 15:01:39 ON 13 NOV 2002)
L25 2 S (L14 OR L10 OR L11 OR L12) AND L19
L26 1 S L25 NOT L17

L26 ANSWER 1 OF 1 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1998:523079 HCAPLUS
DOCUMENT NUMBER: 129:287179
TITLE: A small and efficient catalytic DNA
AUTHOR(S): **Li, Yingfu**; Sen, Dipankar
CORPORATE SOURCE: Institute of Molecular Biology & Biochemistry, Simon Fraser University, BC, V5A 1S6, Can.
SOURCE: Structure, Motion, Interaction and Expression of Biological Macromolecules, Proceedings of the Conversation in the Discipline Biomolecular Stereodynamics, 10th, Albany, June 17-21, 1997 (1998), Meeting Date 1997, Volume 1, 139-149. Editor(s): Sarma, Ramaswamy H.; Sarma, Mukti H. Adenine Press: Schenectady, N. Y.
CODEN: 66NGAV
DOCUMENT TYPE: Conference
LANGUAGE: English
AB A 33-nucleotide, guanine-rich DNA oligomer, PS5.ST1, was selected from a random-sequence DNA library for the property of specifically binding N-methylmesoporphyrin (NMM), a distorted porphyrin that resembles the transition state for the metalation of mesoporphyrin IX by naturally occurring ferrochelatase enzymes. We report that PS5.ST1 is an enzyme (a "DNAzyme"), that catalyzes the insertion of copper and zinc ions into a no. of structurally related porphyrins. This enzyme works with multiple turnovers of substrate, and affords rate accelerations of up to .apprx.3,700 fold over background under optimized conditions. The catalytic efficiency, kcat/KM, which has a value of 4.0 .times. 104 M-1 min-1, is superior to that of a catalytic antibody derived for the same reaction. PS5.M, a 24-nucleotide fragment of PS5.ST1, appears to be the most optimal DNA sequence for this catalysis. PS5.ST1 and PS5.M, both very guanine-rich, require potassium ions for their catalytic activity-consistent the existence of guanine-quartets within their

folded and active structures. Currently, the existence of an array of these biocatalysts, both natural and artificial, for porphyrin metalations permits one-to-one comparisons of the ways in which different biopolymers (proteins, RNA, and DNA) solve a given catalytic problem. Results to date indicate that for porphyrin metalation, RNA and DNA can be quite as **competent** as **proteins** as catalysts.

=> fil hom
FILE 'HOME' ENTERED AT 15:02:44 ON 13 NOV 2002